

# Role of autophagy in the maintenance and function of cancer stem cells

ILIO VITALE<sup>\*,1,2,#</sup>, GWENOLA MANIC<sup>1,#</sup>, VITO DANDREA<sup>3</sup> and RUGGERO DE MARIA<sup>1</sup>

<sup>1</sup>Regina Elena National Cancer Institute, <sup>2</sup>Department of Biology, University of Rome "Tor Vergata" and  
<sup>3</sup>Department of Surgical Sciences, University of Rome "La Sapienza", Rome, Italy

**ABSTRACT** Recent advances in experimental technologies and cancer models have made possible to demonstrate that the tumor is a dynamic system comprising heterogeneous populations of cancer cells organized in a hierarchical fashion with cancer stem cells (CSCs) at the apex. CSCs are immature cells characterized by self-renewal property and long-term repopulation potential. CSCs have been causally linked to cancer initiation, propagation, spreading, recurrence and relapse as well as to resistance to anticancer therapy. A growing body of evidence suggests that the function and physiology of CSCs may be influenced by genetic/epigenetic factors and tumor environment. In this context, macroautophagy is a lysosomal degradative process (herein referred to as autophagy) critical for the adaptive response to stress and the preservation of cellular and tissue homeostasis in all eukaryotes that may have a crucial role of in the origin, maintenance and invasiveness of CSCs. The activation of the autophagic machinery is also considered as an adaptive response of CSCs to perturbation of tumor microenvironment, caused for instance by anticancer therapy. Nevertheless, compelling preclinical and clinical evidence on the cytoprotective role of autophagy for CSCs is still missing. Here, we summarize the results on the contribution of autophagy in CSCs and how it impacts tumorigenesis and tumor progression. We also discuss the therapeutical potential of the modulation of autophagy as a means to eradicate CSCs.

**KEY WORDS:** *autophagosomes, beclin 1, CD133, chloroquine, lysosomes, tumor initiating cells*

## Introduction

It is becoming increasingly evident that solid neoplasms are complex and dynamic entities composed of tumor cells and non-tumor components, including infiltrating endothelial, stromal and immune cells, cancer-associated fibroblasts and constituents of extracellular matrix. The continuous crosstalk between these elements allows for the quick adaptation and rapid response of tumor system to modifications of the environmental conditions such as those provoked for instance by low concentration of oxygen (hypoxia) or nutrient (starvation) as well as anticancer therapy (Hanahan and Coussens, 2012).

As additional layers of complexity, there is compelling evidence demonstrating that malignant tissues display: (1) intertumoral heterogeneity (i.e., few alterations shared by tumors of the same histopathologic subtype) and intratumoral heterogeneity (i.e., distinct phenotypes, genotypes, proliferation rates, metabolisms, metastatic potentials and epigenetic status between neoplastic cells of the same tumor) (De Sousa *et al.*, 2013, Gerlinger *et al.*,

2012); (2) a high frequency of aneuploidy and genetic/chromosomal instability (Gordon *et al.*, 2012, Hanahan and Weinberg, 2011, Vitale *et al.*, 2011a, Vitale *et al.*, 2011b); (3) an extensive metabolic rewiring, which is also believed to accompany neoplastic transformation (Galluzzi *et al.*, 2013, Michels *et al.*, 2015, Schulze and Harris, 2012, Vander Heiden *et al.*, 2009); and (4) a hierarchical organization (Nguyen *et al.*, 2012, Reya *et al.*, 2001). In particular, it has been put in evidence the existence of stem

*Abbreviations used in this paper:* AMPK, AMP-activated protein kinase; ATG, autophagy-related; BCL2, B-cell CLL/lymphoma 2; BECN1, Beclin 1; CML, chronic myeloid leukemia; CQ, chloroquine; CSCs, cancer stem cells; DCIS, ductal carcinoma in situ; DCLK1, doublecortin-like kinase 1; ER, endoplasmic reticulum; LC3, microtubule-associated protein light chain 3; mTOR, mechanistic target of rapamycin (serine/threonine kinase); mTORC1, mTOR complex; PDAC, pancreatic ductal adenocarcinoma; PE, phosphatidylethanolamine; PI3K, phosphatidylinositol-3 kinase; PI3P, phosphatidylinositol-3-phosphate; RB1CC1, RB1-inducible coiled-coil 1; RCD, regulated cell death; shRNAs, short hairpin RNAs; TNBC, triple negative breast cancer; TSC1, tuberous sclerosis 1; ULK, unc-51 like autophagy activating kinase; VPS34, vacuolar protein sorting 34.

\*Address correspondence to: Ilio Vitale. Regina Elena National Cancer Institute, 00144 Roma, Italy. Tel: +39-06-06-5266-2728. E-mail: iliovit@gmail.com

#Note: Both authors equally contributed to this article.

Accepted: 6 May 2015.

cell-like niches, distinct compartments generated within the tumor housing a subpopulation of immature cells known as cancer stem cells (CSCs) (Beck and Blanpain, 2013). CSCs share with stem cells some properties, including the maintenance of an undifferentiated state and the capability of self-renewal upon symmetrical and asymmetrical divisions (Beck and Blanpain, 2013, Kreso and Dick, 2014, Nguyen et al., 2012). These features allow for the perpetuation and the potential expansion of the cancer stem pool as well as the preservation of the stemness potential. Besides this, CSCs are also able to generate non-tumorigenic, daughter cells with variable degree of differentiation. Of note, multilineage differentiation potential is not considered a bona fide stemness property (Kreso and Dick, 2014).

CSCs are believed to drive tumor growth and progression by contributing to the proliferative potential and constituting a source for relapse/recurrence of the disease (Beck and Blanpain, 2013, Greaves, 2013, Kreso and Dick, 2014, Nguyen et al., 2012). These cells have been prospectively isolated by means of specific markers first in acute myeloid leukemia (Bonnet and Dick, 1997, Lapidot et al., 1994) and then in a variety of solid tumors, including breast, brain, colon, lung, pancreas and prostate cancer (Al-Hajj et al., 2003, Collins et al., 2005, Eramo et al., 2008, Hemmati et al., 2003, Hermann et al., 2007, Li et al., 2009b, Patrawala et al., 2006, Ricci-Vitiani et al., 2007, Singh et al., 2004). In these settings, purified CSCs were able to maintain stem cell property and initiate/propagate neoplasms representative of the parental tumors from which they were derived upon serial xenotransplantation into immunodeficient mice.

Recent experimental observations suggest, however, the need of an evolution of the CSC model, as the situation appears more complex than previously thought (Kreso and Dick, 2014, Magee et al., 2012, Meacham and Morrison, 2013, Nguyen et al., 2012, Zeuner et al., 2014b). First, high percentages of CSCs have been shown in some cancers such as acute lymphoblastic leukemia and melanoma (Cojoc et al., 2015, Kong et al., 2008, Quintana et al., 2008). Second, self-renewal is often deregulated in CSCs (Kreso and Dick, 2014). Third, CSCs appear as a heterogeneous population, whose functional properties are influenced by genetic, epigenetic and microenvironmental factors (Baccelli and Trumpp, 2012, Chen and Dent, 2014, Curtis et al., 2010, Dieter et al., 2011, Giannoni et al., 2010, Iliopoulos et al., 2011, Sharma et al., 2010, Suva et al., 2013, Todaro et al., 2014, Vermeulen et al., 2010). Fourth, in some cases, CSCs exist as a dormant and/or quiescent pool potentially contributing to tumor repopulation (Francescangeli et al., 2012, Kreso et al., 2013, Pece et al., 2010, Roesch et al., 2010). Fifth, a certain degree of plasticity in stem cell status has been identified within tumors. For instance, glioblastoma stem cells can differentiate towards neural and mesenchymal lineages (Ricci-Vitiani et al., 2008). Moreover, cancer cells may experience phases of transition between stem-like and non-stem-like states (Chaffer et al., 2011, Chaffer et al., 2013, Gupta et al., 2011, Iliopoulos et al., 2011, Jopling et al., 2011). Thus the process of differentiation seems not to be unidirectional, but rather is based on a dynamic equilibrium between stemness and differentiation.

Despite these novel findings and some contradictory reports (Ishizawa et al., 2010, Quintana et al., 2010, Shackleton et al., 2009), it is generally accepted that CSCs casually contribute to tumor generation and progression in the vast majority of hematological and solid tumors (Baccelli and Trumpp, 2012, Bartucci et

al., 2015; Driessens et al., 2012, Nguyen et al., 2012, Schepers et al., 2012, Todaro et al., 2014). CSCs have clinical relevance as they are endowed with an intrinsic resistance to radio- and chemotherapy (Chen et al., 2012, Cojoc et al., 2015, Eramo et al., 2006, Maugeri-Sacca et al., 2011, Phillips et al., 2006, Vermeulen et al., 2012, Zeuner et al., 2014b). This is believed to occur through mechanisms encompassing the overexpression of one or more members of the ATP-Binding Cassette (ABC) transporter family, which increases drug efflux (Alison et al., 2012); an augmented activity of aldehyde dehydrogenase, which is believed to contribute to the adaptive response to oxidative stress (Ginestier et al., 2007, Kastan et al., 1990); an enhanced activation of DNA damage response often coupled to the evasion of the process of regulated cell death (RCD) (Bao et al., 2006, Bartucci et al., 2012, Capper et al., 2009, Majeti et al., 2009, Todaro et al., 2007, Zeuner et al., 2014a); and the induction of dormancy (Kreso et al., 2013). Emerging evidence also links therapy resistance to the cytoprotective activation of autophagy in CSCs (Biasoli et al., 2013, Chatterjee and van Golen, 2011, Firat et al., 2012, Kantara et al., 2014, Lomonaco et al., 2009, Mai et al., 2012, Ojha et al., 2014, Rao et al., 2012, Wu et al., 2013, Yue et al., 2013). The importance of this catabolic pathway in the function and maintenance of CSCs is however still debated.

Here, we provide an overview of the mechanism of autophagy and the contribution of this process to tumor initiation and progression. We then focus on the role of the autophagic process in CSCs, also discussing the potentiality of strategies based on the modulation of components of the autophagic machinery as a means to efficiently eradicate CSCs.

## Autophagy, a brief overview

### Physiological functions

Autophagy (from the ancient Greek  $\alpha\upsilon\tau\omicron + \phi\alpha\gamma\omicron\sigma/\phi\alpha\gamma\epsilon\acute{\iota}\nu =$  self-eating) is a highly conserved, catabolic process involved in a variety of physiological processes, including normal development, growth and immunity (Choi et al., 2013, Mizushima and Komatsu, 2011, Mizushima et al., 2008). Autophagy deregulation (e.g., owing to defects in autophagy machinery) has been linked to physiological/pathological disorders such as infection, neurodegenerative diseases, cancer and aging (Choi et al., 2013, Levine and Kroemer, 2008, Nixon, 2013, Rubinsztein et al., 2011).

Macroautophagy - the major autophagic pathway in mammals and commonly (and hereafter in this review) referred to as autophagy - is a lysosome-dependent mechanism responsible for the degradation of intracellular components, including cytotoxic protein aggregates, damaged organelles and invading pathogens (Mizushima and Komatsu, 2011, Yang and Klionsky, 2010). During this degradative process, autophagic substrates are normally captured in a non-selective fashion (bulk autophagy). Nevertheless, recent evidence suggests the existence of specialized subtypes of autophagy that selectively recognize their substrates, such as mitophagy, which operates as a mitochondria quality control by eliminating damaged, superfluous or dysfunctional mitochondria (Green and Levine, 2014, Youle and Narendra, 2011).

Autophagy is kept at low level under normal conditions but becomes up-regulated in response to various perturbations, including starvation, hypoxia, pathogen invasion or treatment with cytotoxic compounds (Galluzzi et al., 2014, Kroemer et al., 2010, Mizushima

and Komatsu, 2011). Under these stressful conditions, autophagy is induced to preserve cellular integrity, promote cellular detoxification and provide sources of metabolic energy.

Although the general consensus is that autophagy constitutes a protective response for the short-term adaptation of cells to intracellular stress (Boya *et al.*, 2005, Galluzzi *et al.*, 2012), in some limited instances, such as during the development of *Caenorhadditis elegans* (Erdelyi *et al.*, 2011) and *Drosophila melanogaster* (Berry and Baehrecke, 2007, Denton *et al.*, 2009, Nezis *et al.*, 2010) or upon exposure to chemotherapeutic agents (Grander *et al.*, 2009, Laane *et al.*, 2009, Lamy *et al.*, 2013), this pathway may also mediate cell death. Of note “autophagic cell death” is considered as a type of RCD only when pharmacological or genetic inhibition of the autophagic machinery delays cellular demise (Denton *et al.*, 2012, Galluzzi *et al.*, 2012).

### Molecular mechanisms

Upon its first description, which was possibly made more than 50 years ago when Clark observed the presence of peculiar vesicles containing cytoplasmic organelles by transmission electron microscopy analyses (Clark, 1957), an intense wave of studies have been launched to elucidate the cellular and molecular pathways of autophagy.

The autophagic pathway starts with the sequestration of cytoplasmic material within a double-membraned, non-degradative vesicle known as autophagosome (Lamb *et al.*, 2013). The subsequent fusion of the autophagosome with lysosome generates an autolysosome, the organelle into which the autophagic content is degraded and released into cytoplasm for recycling (Hamasaki *et al.*, 2013, Hayashi-Nishino *et al.*, 2009, Lamb *et al.*, 2013) (Fig. 1).

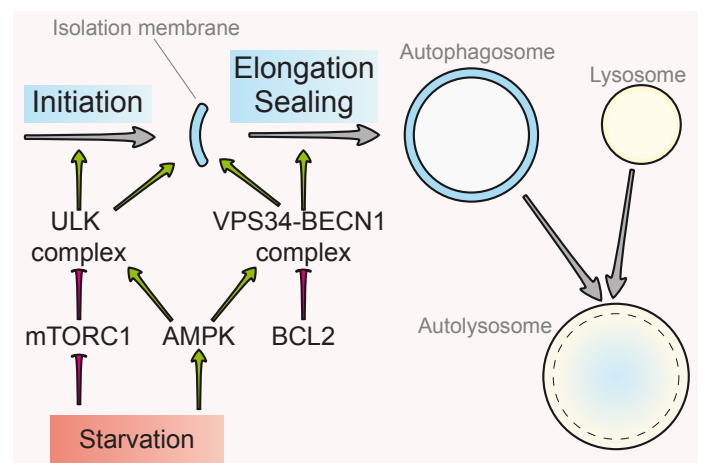
The biogenesis of autophagosomes proceeds via three main steps: 1) the initiation, a signaling pathway whereby the autophagic machinery is targeted to membrane source sites to assembly a pre-autophagosomal membrane, followed by 2) the nucleation of a lipid-based structure known as isolation membrane or phagophore, which is believed to occur at the contact site between endoplasmic reticulum (ER) and mitochondria (Hamasaki *et al.*, 2013, Hayashi-Nishino *et al.*, 2009), and 3) the elongation and sealing of the vesicle to surround autophagic cargoes (Lamb *et al.*, 2013) (Fig. 1). Although the membrane sources for the generation of autophagosome mostly derive from ER and mitochondria, it has been suggested that lipids may also be mobilized to the isolation membrane from plasma membrane (Ravikumar *et al.*, 2010) and other cytoplasmic organelles, including the Golgi (Takahashi *et al.*, 2011).

The molecular core of the autophagic machinery consists of the products of several autophagy-related (*ATG*) genes (Mizushima *et al.*, 2011). *Atg* proteins were first identified in yeast as essential for their survival on nutrient stress and starvation (Thumm *et al.*, 1994, Tsukada and Ohsumi, 1993). The homologues of *ATG* genes have been subsequently identified in almost all organisms, including mammals. So far, at least 19 mammalian *ATG* proteins have been involved in the autophagic process (Choi *et al.*, 2013).

At molecular level autophagosome generation is driven by two main multiprotein complexes, the unc-51 like autophagy activating kinase (ULK) complex and the phosphatidylinositol 3-kinase, catalytic subunit type 3 (PK3C3 best known as vacuolar protein sorting 34, VPS34)-Beclin 1 (BECN1) complex (Wirth *et al.*, 2013). The ULK complex, known as pre-initiation complex, consists of

ULK1, ULK2, RB1-inducible coiled-coil 1 (RB1CC1, best known as FIP200), ATG13 and ATG101 (Ganley *et al.*, 2009, Mizushima, 2010). Both negative and positive regulators of ULK1 complex have been identified. Thus, depending on the energy status and/or nutrient resources of the cells ULK complex may be inhibited or activated by mechanistic target of rapamycin (serine/threonine kinase) (mTOR) complex 1 (mTORC1) or by AMP-activated protein kinase (AMPK), respectively (Hardie *et al.*, 2012, Laplante and Sabatini, 2012). ULK1 associates to the negative regulator mTORC1 under fed condition, while on nutrient depletion mTORC1 becomes inactivated, leading to ULK1 release and autophagy engagement (Hosokawa *et al.*, 2009, Jung *et al.*, 2009). Starvation also results in the activation of AMPK, which triggers autophagy by inhibiting mTORC1 - via AMPK-mediated phosphorylation of the mTORC1 component regulatory associated protein of mTORC1 (RPTOR) (Gwinn *et al.*, 2008) or of tuberous sclerosis 1 (TSC1)-TSC2-TBC1 domain family, member 7 (TBC1D7) (TSC-TBC) complex (Inoki *et al.*, 2003) - or by directly activating ULK1 (Egan *et al.*, 2011, Kim *et al.*, 2011b) (Fig. 1).

The VPS34-BECN1 complex [also known as class III phosphatidylinositol-3 kinase (PI3K) complex] is a regulative platform composed by VPS34, BECN1, autophagy/beclin-1 regulator 1 (AMBRA1) and ATG14L (Funderburk *et al.*, 2010, Yang and Klionsky, 2010). The crucial pro-autophagic event promoted by this complex is the conversion of phosphatidylinositol to phosphatidylinositol-3-phosphate (PI3P) at the site of phagophore nucleation (Wirth *et al.*, 2013). PI3P generation, which is catalyzed by the class III PI3K VPS34 (Meijer and Klionsky, 2011), triggers phagophore nucleation (Axe *et al.*, 2008, Polson *et al.*, 2010). Under normal conditions the anti-apoptotic proteins of the Bcl-2 family B-cell CLL/lymphoma 2 (BCL2) and BCL2-like 1 (BCL2L1, best known as BCLXL) inhibit the VPS34-BECN1 complex by interacting with BECN1, the co-activator of VPS34 (Furuya *et al.*, 2005, Pattingre *et al.*, 2005) (Fig. 1). Such inhibitory association must thus be blocked to promote the PI3K activity of VPS34. The interaction



**Fig. 1. The autophagic pathway.** At cellular level, the two principal steps of autophagy are the generation of an autophagosome from the isolation membrane and its fusion with a lysosome to generate an autolysosome. At molecular level, autophagy relies on the ULK complex and the VPS34-BECN1 complex. The activity of these two macromolecular complexes is finely tuned by both positive (mTORC1 complex) and negative (AMPK and BCL2) regulators.

between BECN1 and BCL2/BCLXL supports the notion of the existence of a complex interplay between autophagy and apoptosis, which appears relevant in the context of cancer and cancer therapy (Marino et al., 2014). On starvation the activation of the VPS34-BECN1 complex is also promoted by AMPK (Kim et al., 2013) or ULK1 (Russell et al., 2013).

Among other molecular components mediating autophagosome generation are included ATG9, a transmembrane protein that is believed to contribute to the recruitment of lipids to the isolation membrane (Mari et al., 2010), and the phosphatidylethanolamine (PE)-conjugated form of microtubule-associated protein light chain 3 (LC3) known as LC3-PE or LC3-II (Weidberg et al., 2010). LC3-II is generated by an ubiquitin-like (UBL) conjugation system via the cleavage of LC3 by the protease ATG4, which produces the cytosolic form LC3-I, followed by the sequential action of the E1-like enzyme ATG7, the E2-like enzyme ATG3 and the E3-ligase complex ATG5-ATG12-ATG16L1, which catalyzes the covalent conjugation of PE to LC3-I (Noda et al., 2008, Ohsumi, 2001). Once generated LC3-II relocates to the nucleation membranes where it exerts a role in the elongation/closure stage (Nakatogawa et al., 2007, Weidberg et al., 2011). Of note, LC3-II remains associated to mature autophagosomal membrane during the autophagic process.

Concerning the last steps of autophagy: the deliver and uptake of the cargoes during autophagosome maturation is mediated by specific adaptors, including the autophagy cargo receptor and substrate p62/sequestosome 1 (SQSTM1, best known as p62), which first associates to proteins or organelles (often tagged by ubiquitin modification, e.g., Lys63 ubiquitination) and then interacts with LC3-like proteins through the LC3-interacting region (LIR) (Birgisdottir et al., 2013, Johansen and Lamark, 2011); the fusion between autophagosome and lysosome is promoted by multiple soluble N-ethylmaleimide-sensitive fusion (NSF) attachment protein receptors-(SNARE-)-like proteins (Fader et al., 2009, Nair et al., 2011). The hydrolysis of the autophagic cargo, including the adaptors, relies on lysosomal enzymes (i.e., acidic hydrolases), whose catabolic activity is triggered by the activation of H<sup>+</sup> pumps, which lowers the pH of the lysosomal lumen (Kroemer et al., 2010). Lysosomal permeases eventually promote the cytosolic export of the product of this degradation for potential reuse (Kuma and Mizushima, 2010).

## Autophagy and cancer

The role of the autophagic machinery in cancer initiation and progression is complex. This catabolic pathway may indeed act either as an oncosuppressive or as a prosurvival mechanism depending on tumor stage and type (White, 2012).

### Autophagy limits the early phases of tumorigenesis

Accumulating evidence suggests that autophagy may act as an antioncogenic barrier by limiting, or even suppressing, cancer initiation. Autophagy is indeed stimulated by tumor suppressors, including phosphatase and tensin homolog (PTEN) or serine/threonine kinase 11 (STK11, also known as LKB1), while being abolished by oncogenic signals, including the overactivation of the PI3K-AKT pathway and the overexpression of the antiapoptotic member of the Bcl-2 family (Morselli et al., 2011). Moreover, an autophagy-dependent anticancer immune response

has been recently reported to control tumorigenesis (Michaud et al., 2011). In addition, mice bearing monoallelic loss of *Becn1* (*Becn1*<sup>+/−</sup>) spontaneously develop tumors (Qu et al., 2003, Yue et al., 2003), while the whole-body absence of ATG4C increases the incidence of chemically-induced tumorigenesis (Marino et al., 2007) and the deletion of *Atg5* or *Atg7* favors the development of liver hepatomas (Takamura et al., 2011). Along similar lines, the monoallelic deletion of *BECN1* has been described in a large fraction of human neoplasms, including breast, ovarian and prostate cancer (Aita et al., 1999, Choi et al., 2013, Liang et al., 1999), while mutations of other autophagic proteins (e.g., *ATG5*, *ATG12*) have been found in colorectal cancers (Kang et al., 2009). Altered expression of autophagic proteins has also been detected in some tumors. For instance low BECN1 levels correlated with poor prognosis in multiple human malignancies including, colorectal, lung, esophageal and pancreatic cancer (Chen et al., 2009, Jiang et al., 2012, Kim et al., 2011a, Li et al., 2009a), while high BECN1 levels have been associated with improved survival in patients affected by high-grade gliomas, hepatocellular carcinoma or large B-cell lymphoma (Ding et al., 2008, Huang et al., 2011, Pirtoli et al., 2009). In addition, both ATG5 and LC3 levels were found decreased in patients with melanomas as compared with those with benign nevi (Liu et al., 2013).

Despite some contradictory observations (Kim et al., 2011c, Laddha et al., 2014, Takamura et al., 2011, Wan et al., 2010, Wei et al., 2011) the current hypothesis pleads in favor of an oncosuppressive role for autophagy. According to this model, the suppression of this catabolic process would promote oncogenesis by increasing the level of oxidative stress (White, 2012, Yang et al., 2011a); inducing genomic instability (due to accumulation of genomic alteration and/or micronuclei) (Mathew et al., 2007, Rello-Varona et al., 2012, Xie et al., 2011); rewiring bioenergetic metabolism (due to a defective mitochondria turnover) (Karantza-Wadsworth et al., 2007) counteracting oncogene-induced senescence (Iannello and Raulet, 2014, Iannello et al., 2013, Liu et al., 2013, Xue et al., 2007, Young et al., 2009); favoring the accumulation of p62, which triggers a pro-tumorigenic signal via the antioxidant transcription factor nuclear factor, erythroid 2-like 2 (NFE2L2, best known as NRF2) (Inami et al., 2011, Jain et al., 2010); and affecting the mechanism of antitumor immunosurveillance (due to perturbations in the tumor microenvironment) (Michaud et al., 2011, Rao et al., 2014).

### Autophagy promotes the survival of cancer cells

A growing body of evidence suggests that once established tumors retain and/or activate autophagy (or reinstate it if the autophagic machinery has been disabled in the early phase of oncogenesis) for sustaining their progression. Human tumor cell lines, and in particular RAS-driven pancreatic cancer cells, display indeed an upregulated basal level of autophagy as compared to normal cells, and this has been associated to poor outcome and increased tumor survival (Guo et al., 2011, Lazova et al., 2012, Wang et al., 2012, Yang et al., 2011a). In addition, autophagy is induced as a cancer pro-survival pathway in response to multiple stress conditions in tumor microenvironment including starvation and hypoxia (Amaravadi et al., 2011, Kimmelman, 2011, White, 2012).

Some experimental evidence supports the notion of a pro-oncogenic function of autophagy in established cancer. First, the hemizygous deletion of *Becn1* hampers tumor formation in ataxia

telangiectasia mutated (*Atm*)<sup>-/-</sup> and *Tsc2*<sup>-/-</sup> mice (Parkhitko *et al.*, 2011). Apparently at odds with this, enhanced BECN1 inactivation augments tumor growth of established non-small-cell lung cancer with active mutation of epidermal growth factor (EGFR) (Wei *et al.*, 2013b). Second, the ablation of *Rb1cc1* limits tumor progression in a mouse model of breast cancer driven by the polyoma middle T oncogene (PyMT) oncogene (Wei *et al.*, 2011). Third, the hepatic-specific deletion of *Atg5* or *Atg7* arrests the transition from hepatoma to hepatocellular carcinoma in genetically modified mouse models (Takamura *et al.*, 2011). Fourth, disabling autophagy limits tumor growth in KRAS-transformed cells by interfering with the metabolic functions and the turnover of mitochondria, and consequently affecting both the level of metabolic/biosynthetic substrates and the energy charge (Guo *et al.*, 2011). Fifth, autophagy is reported to facilitate mammary tumorigenesis and promote tumor growth in a model of partner and localizer of breast cancer 2, early onset (BRCA2)-(PALB2)-associated hereditary breast cancer (Huo *et al.*, 2013). Sixth, studies performed on genetically engineered mouse models of KRAS<sup>G12D</sup>- or BRAF<sup>V600E</sup>-driven lung cancer suggest that autophagy dictates lung tumor fate (Chen and Guan, 2013, Guo *et al.*, 2013, Rao *et al.*, 2014). Thus, tissue-specific inactivation of ATG5 or ATG7 respectively reduced the progression from adenomas to adenocarcinomas (Rao *et al.*, 2014) or diverted it to more benign tumor oncocytomas (Chen and Guan, 2013, Guo *et al.*, 2013). Of note, the negative impact of autophagy inhibition on tumor progression could be in part reverted by the deletion of the transformation related protein 53 (Trp53, best known as p53). Seventh, in a humanized genetically-modified mouse model of KRAS<sup>G12D</sup>-driven pancreatic ductal adenocarcinoma (PDAC), the absence of either ATG5 or ATG7 blocks tumor progression to PDAC (Rosenfeldt *et al.*, 2013). Intriguingly, in this study the combination of autophagy loss and p53 deficiency accelerated tumor initiation and progression, suggesting that p53 status may dictate the role of autophagy in pancreatic tumor development.

Altogether these observations suggest that autophagy promotes the survival of cancer cell by favoring their adaptation and tolerance to environmental stress such as hypoxia, by maintaining mitochondrial homeostasis and functions (e.g., by providing substrates for mitochondrial metabolisms via nutrient recycling or limiting the accumulation of ROS), and by affecting p53 tumor suppressor pathway.

### Autophagy in the physiology of CSCs

An increasing number of experimental observations suggest that autophagy may be crucial in the maintenance and function of distinct types of normal stem cells. This catabolic process is indeed involved in the preservation of stem cell homeostasis and the maintenance of stemness property (reviewed in (Guan *et al.*, 2013, Phadwal *et al.*, 2013, Vessoni *et al.*, 2012)). Of note, the protein/organelle quality control operated by autophagy appears of particular relevance during periods of quiescence and/or differentiation (Guan *et al.*, 2013). The exact contribution of autophagy for the biology of CSCs is not yet elucidated (Lin *et al.*, 2015). Given the analogy between CSCs and stem cells, one could expect that autophagy may play a cytoprotective role for CSCs. Nevertheless, as autophagy suppresses the early phases of tumorigenesis (see above), a deregulation of this catabolic process in CSCs could also be plausible. To further complicate this

“autophagy paradox”, the mechanism of autophagic cell death may be relevant for the effectiveness of specific antineoplastic therapies. In the next paragraphs we summarize the experimental evidence available on the contribution of autophagy in CSCs.

### Autophagy promotes CSCs survival

Some components of autophagic machinery are essential for the survival of CSCs and their adaptation to changes of the tumor microenvironment. This applies to: 1) ATG4A, which has been identified as a pivotal factor for CSC maintenance in a high-throughput screening based on mammosphere formation upon transfection with a pool of short hairpin RNAs (shRNAs) (Wolf *et al.*, 2013); 2) BECN1, which has been shown to promote the survival of breast CSCs (but not bulk cancer cells) and to contribute to their *in vivo* tumorigenicity (Gong *et al.*, 2013). In this experimental setting, the expression level of BECN1, and consequently the autophagic flux, were indeed higher in CSC-enriched than in non-CSC-enriched breast tumor cells; 3) DRAM1 and p62, which have been found overexpressed in adult glioblastoma tumors belonging to the mesenchymal subtype (Galavotti *et al.*, 2013). In this study, by using specific siRNAs directed against DRAM1 and p62 both proteins were mechanistically linked to the regulation of bioenergetic metabolism, migration and invasion of glioblastoma CSCs.

Further confirming the crucial role of autophagy in CSC maintenance, the stable or specific knockdown of LC3 and ATG12 (by means of lentiviral-delivered shRNAs) decreased the fraction of tumor cells expressing high levels of CD44 and low levels of CD24 antigens (CD44<sup>+</sup>/CD24<sup>-low</sup>), which are believed to include the population of breast CSCs (Cufi *et al.*, 2011). Accordingly, an elevated autophagic flux was found in mammospheres derived from triple negative breast cancer (TNBC) as compared to parental cells (Rao *et al.*, 2012). Moreover, it has been recently demonstrated that a subpopulation of human pancreatic CSCs resistant to KRAS ablation and responsible for tumor relapse relied on autophagy and other catabolic processes for its survival (Viale *et al.*, 2014). Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells harboring the chimeric gene *BCR-ABL*. Both the survival of CML cells and leukemogenesis was shown to strictly rely on autophagy, as demonstrated in experiments performed by using the conditional knockout of *ATG3* (Altman *et al.*, 2011). The survival of CML stem/progenitor cells was also impaired by the depletion of *ATG4B* (Rothe *et al.*, 2014). Along similar lines, the inhibition of autophagy via the administration of chloroquine (CQ, a lysosomotropic agent arresting the fusion between autophagosomes and lysosomes (Firat *et al.*, 2012, O'Donovan *et al.*, 2011, Sasaki *et al.*, 2010, Selvakumaran *et al.*, 2013)) depleted the CD44<sup>+</sup>/CD24<sup>-low</sup> population in TNBC, both in preclinical and clinical settings (Choi *et al.*, 2014). CQ also abolished the propagation, invasion and growth of fresh spheroid-forming cells derived from human ductal carcinoma *in situ* (DCIS), the most common non-invasive, pre-malignant condition often progressing to invasive breast cancer (Espina *et al.*, 2010). It should be noted, however, that CQ may modulate signaling pathways other than autophagy, including the permeabilization of lysosomal membrane and the subsequent activation of the mitochondrial pathway of apoptosis (Maycotte *et al.*, 2012, Rubinshtein *et al.*, 2012). Moreover, CQ is reported to exert anti-CSC activity through autophagy-unrelated mechanisms, including the inhibition of Janus kinase 2 (JAK2) (Choi *et al.*, 2014) or the inactivation of the CXCR4 and Hedgehog signaling (Balic *et al.*, 2014).

CD133 (also known as prominin-1) was one of the first surface markers used to prospectively purify and thus enrich CSCs (Singh *et al.*, 2004). Despite some contentions (Clement *et al.*, 2009, Wang *et al.*, 2008), CD133 has been proposed as a marker of a number of solid tumors, and its expression is predictive of glioma patient outcome (Pallini *et al.*, 2008). CD133 has also been described as a marker of bioenergetic stress (Griguer *et al.*, 2008). In line with this, two recent reports of the same group described a prosurvival role of CD133 in hepatoma cancer exerted through CD133-mediated activation of autophagy (Chen *et al.*, 2013a, Chen *et al.*, 2013b). In the first study, the authors showed that a CD133 antibody inhibited spheroids and xenograft tumor formation in NOD/SCID mice, and induced cancer cell death by suppressing autophagy and promoting necrotic cell death (Chen *et al.*, 2013b). In the second study, they reported that on glucose deprivation CD133 translocated to cytoplasm where it promotes autophagosome formation, glucose uptake and ATP synthesis by a mechanism not fully elucidated (Chen *et al.*, 2013a). In this latter experimental setting, the depletion of CD133 also reduced xenograft tumor formation. Apparently at odd with these observations, CD133 is described to activate the PI3K/AKT/mTOR pathway (which among other functions also inhibits autophagy, see above) by interacting with PI3K, and its depletion reduced the self-renewal and tumorigenicity of glioblastoma CSCs (Wei *et al.*, 2013a). This is in line with a role of the PI3K/AKT/mTOR signaling in the origin, survival and maintenance of CSCs from both hematological and solid tumors (Chang *et al.*, 2013, Dubrovskaya *et al.*, 2009, Francipane and Lagasse, 2013, Martelli *et al.*, 2011, Zhou *et al.*, 2007). Moreover, Cho and colleagues reported that the expression of sex determining region Y (SRY)-box 2 (*SOX2*), a transcription factor involved in the regulation of embryonic development with essential roles for stem-cell maintenance and pluripotency (Boyer *et al.*, 2005), triggered the activation of either autophagy (by inducing the overexpression of *ATG10*) or cellular senescence in colon cancer cells, in turn resulting in the arrest of tumor growth, both *in vitro* and *in vivo* (Cho *et al.*, 2013).

The current view is that autophagy acts as a pro-survival mechanism whereby CSCs face changes in the tumor microenvironment. Reportedly, extrinsic perturbations, including hypoxic, metabolic and oxidative stress, can promote self-renewal and plasticity. For instance, in hypoxic conditions (such as those encountered in the hypoxic niche (Mohyeldin *et al.*, 2010)) hypoxia inducible factor (HIF) mediates the activation of stem cell markers/factors as well as prosurvival pathways, including autophagy (Heddleston *et al.*, 2009, Lin and Yun, 2010, Ma *et al.*, 2011, Mathieu *et al.*, 2011, Qiang *et al.*, 2012). Accordingly, a high number of autophagosomes have been reported in poorly vascularized, hypoxic tumor regions (Degenhardt *et al.*, 2006). Of note, HIF-inducing autophagy has been recently shown to promote the metastatic ability of CD133+ pancreatic cancer stem cells (Zhu *et al.*, 2014). In addition, antiangiogenic agents increased the population of CSCs on hypoxia and this phenomenon could limit their anticancer activity (Conley *et al.*, 2012). In line with the hypothesis of a role of autophagy in the adaptation of CSCs to environmental perturbations, interfering with autophagic flux decreased the survival of either pancreatic cancer cells with stem-like properties under hypoxic conditions (Rausch *et al.*, 2012) or CD133+ liver CSCs on oxygen- and nutrient-deprivation (Song *et al.*, 2013). Importantly, a co-expression between hypoxia, stemness and autophagy

markers was found in immunohistochemical analyses performed on tissue samples from PDAC patients (Rausch *et al.*, 2012).

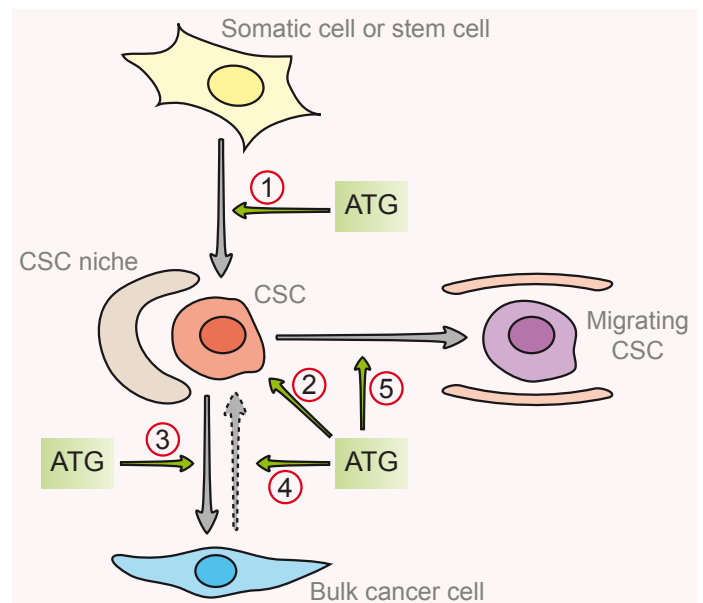
Finally, intriguing evidence also ascribes to autophagy a role in CSC plasticity. Thus, the inhibition of mTOR (and the subsequent induction of autophagy, Fig. 1) increased the CD133+ fraction in liver tumor cells by arresting the differentiation and inducing the conversion of CD133- to CD133+ cells and also promoted *in vivo* tumor growth (Yang *et al.*, 2011b). Similar results were found by Zhu and collaborators, who demonstrated that the activation of autophagy under hypoxic conditions promoted the dedifferentiation of non-stem pancreatic cancer cell population into stem-like cells (Zhu *et al.*, 2013).

Taken together, these observations suggest that the cytoprotective role of autophagy for CSCs has a crucial impact on tumor initiation, progression and spreading (Fig. 2). Nevertheless, compelling clinical evidence on the exact contribution of autophagy to CSC physiology is required, as in some settings autophagy activation (for instance via the inhibition of the PI3K/AKT/mTOR pathway) may also exert antineoplastic activity by targeting CSCs.

#### Autophagy activation in CSCs affects therapy response

Contrasting results link the autophagic process to either the resistance of CSCs to, or the fully execution of cell death induced by, radio or chemotherapy.

On the one hand, the depletion of autophagy-related genes and/or the pharmacological inhibition of autophagy increased the sensitivity of glioma CSCs to radiation therapy (Firat *et al.*, 2012, Lomonaco *et al.*, 2009). In one of these two studies, the simultaneous administration of the inhibitor of the PI3K/AKT pathway boosted the antitumor efficacy and allowed for CQ dose reduction (Firat *et al.*, 2012). Salinomycin is a compound identi-



**Fig. 2. Contribution of autophagy to cancer stem cell (CSC) biology.** Recent evidence suggests that the activation of the autophagy (ATG) contributes to the generation (1), survival (2), differentiation (3), plasticity (4) and migrating/invasion property (5) of CSCs. The impact of the autophagy process on CSC biology may thus have a direct role on tumor initiation, progression and metastasis.

fied in a screening for novel agents specifically killing epithelial CSCs (Gupta *et al.*, 2009). The molecular mechanism underlying this anti-CSCs effect has been recently characterized to involve the direct abrogation of autophagy by salinomycin (Yue *et al.*, 2013). In addition, the knockdown of ATG7 potentiated the inhibitory effect of salinomycin on breast CSC survival and expansion (Yue *et al.*, 2013). Along similar lines, the inhibition of the autophagic flux increased the killing effect of etoposide in glioblastoma cell lines and CSCs (Biasoli *et al.*, 2013), sensitized breast CSCs to ginsenoside F2 (Mai *et al.*, 2012), and inhibited mammosphere formation of TNBC cells, while enhancing the *in vitro* and *in vivo* anticancer efficacy of panobinostat (Rao *et al.*, 2012). Moreover, autophagy promoted the resistance of colon CSCs to paclitaxel by inhibiting apoptosis and upregulating caudal type homeobox 1 (CDX1) (Wu *et al.*, 2013), induced dormancy in breast CSCs exposed to farnesyl-transferase inhibitors (Chatterjee and van Golen, 2011), and protected bladder cancer side population (SP) (Golebiewska *et al.*, 2011) cells from chemotherapeutic agents such as gemcitabine, mitomycin and cisplatin (Ojha *et al.*, 2014). In addition, curcumin enhanced the proliferation and autophagic survival of doublecortin-like kinase 1 (DCLK1)-positive colon CSCs (Kantara *et al.*, 2014). This finding supports the use of DCLK1 as a potential target to sensitize tumors to curcumin. Finally, the depletion of autophagy by different approaches increased the cytotoxicity of the tyrosine kinase inhibitor imatinib or the AKT inhibitor perifosine (two drugs reported to activate autophagy) in CML cell lines (Bellodi *et al.*, 2009, Elzinga *et al.*, 2013, Rothe *et al.*, 2014, Tong *et al.*, 2012, Yu *et al.*, 2012).

On the other hand, the cytotoxic effect of some therapeutic agents is mediated by (and strictly requires) the molecular machinery of autophagy. This applies to 1) temozolomide, whose effect on glioblastoma CSCs involves the activation of autophagic cell death, suggesting that the down-regulation of autophagy-related proteins may be a mechanism to evade temozolomide-induced cytotoxicity (Fu *et al.*, 2009); 2) resveratrol, which eliminates breast CSCs by inducing autophagy via the suppression of the Wnt- $\beta$  catenin signaling pathway (Fu *et al.*, 2014) or upstream of the activation of apoptosis (Filippi-Chiela *et al.*, 2011) and 3) metformin (a pharmacological agent currently employed for the treatment of type 2 diabetes and known to selectively kill breast CSCs (Hirsch *et al.*, 2009)), which seems to exert its anti-CSC effect by modulating the mTOR signaling pathway (Mohammed *et al.*, 2013). Along similar line, the depletion of DNA-PKcs radiosensitized glioma CSCs by inducing autophagic cell death (Zhuang *et al.*, 2011b), while autophagy activation by the modulation of mTOR activity promoted neuroblastoma and glioma stem cell differentiation and abrogated resistance of glioma stem cells to radiation (Zeng and Zhou, 2008, Zhao *et al.*, 2010, Zhuang *et al.*, 2011a, Zhuang *et al.*, 2011c). Finally, brain CSCs succumbed to adenovirus-mediated cell death via autophagy, both *in vitro* and *in vivo* (Jiang *et al.*, 2007).

Reportedly, some drugs may simultaneously trigger distinct pathways of RCD (Galluzzi *et al.*, 2015, Galluzzi *et al.*, 2012). For instance rotterin promotes autophagy followed by cell death in breast, pancreatic or prostate CSCs by acting on the PI3K/AKT/mTOR cascade (Francipane and Lagasse, 2013, Kumar *et al.*, 2013, Kumar *et al.*, 2014, Singh *et al.*, 2012). Rotterin-induced autophagy may thus act as a survival mechanism limiting apoptosis or, alternatively, contribute to the activation of this RCD

subroutine. Finally, in one report, no significant difference was observed in the induction of apoptosis and autophagic cell death between CD44<sup>+</sup>/CD24<sup>-low</sup> breast CSCs and parental cells (Yenigun *et al.*, 2013). Altogether, these findings suggest that autophagy inhibition and autophagy activation may be both considered as promising strategies for sensitizing CSCs to anticancer therapy.

## Concluding remarks

The contribution of autophagy in the physiology of CSCs appears complex and is not yet fully elucidated. Accumulating evidence suggests that the autophagic process actively contributes to the generation, maintenance, plasticity, distribution and migratory/invasion potential of CSCs (Fig. 2). Moreover this catabolic process takes part to the adaptive stress response mounted by CSCs to cope with perturbations of tumor microenvironment (e.g., hypoxia or therapy).

The inhibition of prosurvival pathways preferentially activated in (and presumably strictly required for the survival of) CSCs, including Notch (McAuliffe *et al.*, 2012, Takebe *et al.*, 2011, Ulasov *et al.*, 2011), Sonic Hedgehog (Song *et al.*, 2011, Takebe *et al.*, 2011, Ulasov *et al.*, 2011), Wnt- $\beta$  catenin (Kendziorra *et al.*, 2011, Takebe *et al.*, 2011) and NF- $\kappa$ B (Garner *et al.*, 2013, Sun *et al.*, 2013) signaling cascades, is considered as an efficient antineoplastic strategy. By using a similar approach, the components of the autophagic machinery may thus be employed as a promising target to selectively eradicate CSCs thereby arresting tumor growth/progression/spreading and improving the effectiveness of radio- and chemotherapy (which both are reported to enrich CSCs in tumors) (Bao *et al.*, 2006, De Sousa *et al.*, 2013, Phillips *et al.*, 2006).

Nevertheless, some evidence suggests caution in the use of autophagy inhibitors for cancer therapy: (1) autophagy has a physiological role in the preservation of tissue homeostasis, is involved in innate/adaptive immunity and also acts as a barrier against tumorigenesis and neurodegenerative diseases, implying that its whole-body inhibition may have adverse effects (Choi *et al.*, 2013); (2) CQ and hydroxychloroquine (i.e., the drugs currently used for inhibiting autophagy in multiple ongoing clinical trials launched on cancer patients, source: <http://clinicaltrials.gov/>) (Manic *et al.*, 2014) have activities on lysosomal (and possibly non-lysosomal) processes distinct from autophagy (Balci *et al.*, 2014, Choi *et al.*, 2014, Maycotte *et al.*, 2012, Rubinsztein *et al.*, 2012) suggesting the need to develop novel, specific pharmacological inhibitors of autophagy; (3) autophagic proteins have autophagy-independent roles (for instance BECN1 contributes to vesicular trafficking pathways (Cao and Klionsky, 2007, Shrivage *et al.*, 2013); (4) in some cases autophagy drives the anticancer and anti-CSC activity of specific antitumor agents (Filippi-Chiela *et al.*, 2011, Fu *et al.*, 2009, Fu *et al.*, 2014, Mohammed *et al.*, 2013, Zhuang *et al.*, 2011b), meaning that autophagy inhibition may lead to therapeutic failure; and (5) some signaling pathways that de facto inhibit the molecular machinery of autophagy (e.g. the PI3K/AKT/mTOR cascade) are described as cytoprotective for CSCs (Chang *et al.*, 2013, Dubrovskaya *et al.*, 2009, Martelli *et al.*, 2011), suggesting that the activation of the autophagic process may also be a potential means to kill cancer cells including CSCs. The real impact of autophagy in CSCs may thus depend on the type of tumor, stage of tumorigenesis, tumor microenvironment

as well as the genetic, epigenetic and metabolic context.

Uncovering the exact contribution of autophagy in tumor system and CSC biology, and the specific role of this catabolic pathway in CSCs may be crucial for the development of novel antineoplastic therapy aiming at tumor eradication.

#### Acknowledgements

G.M. is supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC). I.V. is funded by AIRC (MFAG 14641), the Italian Ministry of Health (RF\_GR 2011-2012) and the Programma per i Giovani Ricercatori "Rita Levi Montalcini" 2011. This work was also supported by the Italian Association for Cancer Research (AIRC) with a "5 per mille" grant to R.D.M.

#### References

- AITA, V.M., LIANG, X.H., MURTY, V.V., PINCUS, D.L., YU, W., CAYANIS, E., KALACHIKOV, S., GILLIAM, T.C. and LEVINE, B. (1999). Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. *Genomics* 59: 59-65.
- AL-HAJJ, M., WICHA, M.S., BENITO-HERNANDEZ, A., MORRISON, S.J. and CLARKE, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 100: 3983-3988.
- ALISON, M.R., LIN, W.R., LIM, S.M. and NICHOLSON, L.J. (2012). Cancer stem cells: in the line of fire. *Cancer Treat Rev* 38: 589-598.
- ALTMAN, B.J., JACOBS, S.R., MASON, E.F., MICHALEK, R.D., MACINTYRE, A.N., COLOFF, J.L., ILKAYEVA, O., JIA, W., HE, Y.W. and RATHMELL, J.C. (2011). Autophagy is essential to suppress cell stress and to allow BCR-Abl-mediated leukemogenesis. *Oncogene* 30: 1855-1867.
- AMARAVADI, R.K., LIPPINCOTT-SCHWARTZ, J., YIN, X.M., WEISS, W.A., TAKEBE, N., TIMMER, W., DIPAOLO, R.S., LOTZE, M.T. and WHITE, E. (2011). Principles and current strategies for targeting autophagy for cancer treatment. *Clin Cancer Res* 17: 654-666.
- AXE, E.L., WALKER, S.A., MANIFAVA, M., CHANDRA, P., RODERICK, H.L., HABERMANN, A., GRIFFITHS, G. and KTIKISTAKIS, N.T. (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* 182: 685-701.
- BACCELLI, I. and TRUMPP, A. (2012). The evolving concept of cancer and metastasis stem cells. *J Cell Biol* 198: 281-293.
- BALIC, A., SORENSEN, M.D., TRABULO, S.M., SAINZ, B., JR., CIOFFI, M., VIEIRA, C.R., MIRANDA-LORENZO, I., HIDALGO, M., KLEEFF, J., ERKAN, M. et al., (2014). Chloroquine targets pancreatic cancer stem cells via inhibition of CXCR4 and hedgehog signaling. *Mol Cancer Ther* 13: 1758-1771.
- BAO, S., WU, Q., MCLENDON, R.E., HAO, Y., SHI, Q., HJELMELAND, A.B., DEWHIRST, M.W., BIGNER, D.D. and RICH, J.N. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444: 756-760.
- BARTUCCI, M., DATTILO, R., MORICONI, C., PAGLIUCA, A., MOTTOLESE, M., FEDERICI, G., BENEDETTO, A.D., TODARO, M., STASSI, G., SPERATI, F. et al., (2015). TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. *Oncogene* 34: 681-690.
- BARTUCCI, M., SVENSSON, S., ROMANIA, P., DATTILO, R., PATRIZII, M., SIGNORE, M., NAVARRA, S., LOTTI, F., BIFFONI, M., PILOZZI, E. et al., (2012). Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy. *Cell Death Differ* 19: 768-778.
- BECK, B. and BLANPAIN, C. (2013). Unravelling cancer stem cell potential. *Nat Rev Cancer* 13: 727-738.
- BELLODI, C., LIDONNICI, M.R., HAMILTON, A., HELGASON, G.V., SOLIERA, A.R., RONCHETTI, M., GALAVOTTI, S., YOUNG, K.W., SELMI, T., YACOBI, R. et al., (2009). Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest* 119: 1109-1123.
- BERRY, D.L. and BAEHRECKE, E.H. (2007). Growth arrest and autophagy are required for salivary gland cell degradation in *Drosophila*. *Cell* 131: 1137-1148.
- BIASOLI, D., KAHN, S.A., CORNELIO, T.A., FURTADO, M., CAMPANATI, L., CHNEIWEISS, H., MOURA-NETO, V. and BORGES, H.L. (2013). Retinoblastoma protein regulates the crosstalk between autophagy and apoptosis, and favors glioblastoma resistance to etoposide. *Cell Death Dis* 4: e767.
- BIRGISDOTTIR, A.B., LAMARK, T. and JOHANSEN, T. (2013). The LIR motif - crucial for selective autophagy. *J Cell Sci* 126: 3237-3247.
- BONNET, D. and DICK, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3: 730-737.
- BOYA, P., GONZALEZ-POLO, R.A., CASARES, N., PERFETTINI, J.L., DESSEN, P., LAROCLETTE, N., METIVIER, D., MELEY, D., SOUQUERE, S., YOSHIMORI, T. et al., (2005). Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* 25: 1025-1040.
- BOYER, L.A., LEE, T.I., COLE, M.F., JOHNSTONE, S.E., LEVINE, S.S., ZUCKER, J.P., GUENTHER, M.G., KUMAR, R.M., MURRAY, H.L., JENNER, R.G. et al., (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 122: 947-956.
- CAO, Y. and KLIONSKY, D.J. (2007). Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. *Cell Res* 17: 839-849.
- CAPPER, D., GAISER, T., HARTMANN, C., HABEL, A., MUELLER, W., HEROLD-MENDE, C., VON DEIMLING, A. and SIEGELIN, M.D. (2009). Stem-cell-like glioma cells are resistant to TRAIL/Apo2L and exhibit down-regulation of caspase-8 by promoter methylation. *Acta Neuropathol* 117: 445-456.
- CHAFFER, C.L., BRUECKMANN, I., SCHEEL, C., KAESTLI, A.J., WIGGINS, P.A., RODRIGUES, L.O., BROOKS, M., REINHARDT, F., SU, Y., POLYAK, K. et al., (2011). Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci USA* 108: 7950-7955.
- CHAFFER, C.L., MARJANOVIC, N.D., LEE, T., BELL, G., KLEER, C.G., REINHARDT, F., D'ALESSIO, A.C., YOUNG, R.A. and WEINBERG, R.A. (2013). Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell* 154: 61-74.
- CHANG, L., GRAHAM, P.H., HAO, J., NI, J., BUCCI, J., COZZI, P.J., KEARSLEY, J.H. and LI, Y. (2013). Acquisition of epithelial-mesenchymal transition and cancer stem cell phenotypes is associated with activation of the PI3K/Akt/mTOR pathway in prostate cancer radioresistance. *Cell Death Dis* 4: e875.
- CHATERJEE, M. and VAN GOLEN, K.L. (2011). Breast cancer stem cells survive periods of farnesyl-transferase inhibitor-induced dormancy by undergoing autophagy. *Bone Marrow Res* 2011: 362938.
- CHEN, H., LUO, Z., DONG, L., TAN, Y., YANG, J., FENG, G., WU, M., LI, Z. and WANG, H. (2013a). CD133/prominin-1-mediated autophagy and glucose uptake beneficial for hepatoma cell survival. *PLoS One* 8: e56878.
- CHEN, H., LUO, Z., SUN, W., ZHANG, C., SUN, H., ZHAO, N., DING, J., WU, M., LI, Z. and WANG, H. (2013b). Low glucose promotes CD133mAb-elicited cell death via inhibition of autophagy in hepatocarcinoma cells. *Cancer Lett* 336: 204-212.
- CHEN, J., LI, Y., YU, T.S., MCKAY, R.M., BURNS, D.K., KERNIE, S.G. and PARADA, L.F. (2012). A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* 488: 522-526.
- CHEN, S. and GUAN, J.L. (2013). Tumor-promoting and -suppressive roles of autophagy in the same mouse model of BrAfv600E-driven lung cancer. *Cancer Discov* 3: 1225-1227.
- CHEN, T. and DENT, S.Y. (2014). Chromatin modifiers and remodellers: regulators of cellular differentiation. *Nat Rev Genet* 15: 93-106.
- CHEN, Y., LU, Y., LU, C. and ZHANG, L. (2009). Beclin-1 expression is a predictor of clinical outcome in patients with esophageal squamous cell carcinoma and correlated to hypoxia-inducible factor (HIF)-1 $\alpha$  expression. *Pathol Oncol Res* 15: 487-493.
- CHO, Y.Y., KIM, D.J., LEE, H.S., JEONG, C.H., CHO, E.J., KIM, M.O., BYUN, S., LEE, K.Y., YAO, K., CARPER, A. et al., (2013). Autophagy and cellular senescence mediated by Sox2 suppress malignancy of cancer cells. *PLoS One* 8: e57172.
- CHOI, A.M., RYTER, S.W. and LEVINE, B. (2013). Autophagy in human health and disease. *N Engl J Med* 368: 651-662.
- CHOI, D.S., BLANCO, E., KIM, Y.S., RODRIGUEZ, A.A., ZHAO, H., HUANG, T.H., CHEN, C.L., JIN, G., LANDIS, M.D., BUREY, L.A. et al., (2014). Chloroquine Eliminates Cancer Stem Cells Through Dereglulation of Jak2 and DNMT1. *Stem Cells* 32: 2309-2323.
- CLARK, S.L., JR. (1957). Cellular differentiation in the kidneys of newborn mice studies with the electron microscope. *J Biophys Biochem Cytol* 3: 349-362.
- CLEMENT, V., DUTOIT, V., MARINO, D., DIETRICH, P.Y. and RADOVANOVIC, I. (2009). Limits of CD133 as a marker of glioma self-renewing cells. *Int J Cancer*



- 125: 244-248.
- COJOC, M., MABERT, K., MUDERS, M.H. and DUBROVSKA, A. (2015). A role for cancer stem cells in therapy resistance: Cellular and molecular mechanisms. *Semin Cancer Biol.* 31: 16-27.
- COLLINS, A.T., BERRY, P.A., HYDE, C., STOWER, M.J. and MAITLAND, N.J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 65: 10946-10951.
- CONLEY, S.J., GHEORDUNESCU, E., KAKARALA, P., NEWMAN, B., KORKAYA, H., HEATH, A.N., CLOUTHIER, S.G. and WICHA, M.S. (2012). Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc Natl Acad Sci USA* 109: 2784-2789.
- CUFI, S., VAZQUEZ-MARTIN, A., OLIVERAS-FERRAROS, C., MARTIN-CASTILLO, B., VELLON, L. and MENENDEZ, J.A. (2011). Autophagy positively regulates the CD44(+) CD24(-/low) breast cancer stem-like phenotype. *Cell Cycle* 10: 3871-3885.
- CURTIS, S.J., SINKEVICIUS, K.W., LI, D., LAU, A.N., ROACH, R.R., ZAMPONI, R., WOOLFENDEN, A.E., KIRSCH, D.G., WONG, K.K. and KIM, C.F. (2010). Primary tumor genotype is an important determinant in identification of lung cancer propagating cells. *Cell Stem Cell* 7: 127-133.
- DE SOUSA, E.M.F., VERMEULEN, L., FESSLER, E. and MEDEMA, J.P. (2013). Cancer heterogeneity--a multifaceted view. *EMBO Rep* 14: 686-695.
- DEGENHARDT, K., MATHEW, R., BEAUDOIN, B., BRAY, K., ANDERSON, D., CHEN, G., MUKHERJEE, C., SHI, Y., GELINAS, C., FAN, Y. *et al.*, (2006). Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 10: 51-64.
- DENTON, D., NICOLSON, S. and KUMAR, S. (2012). Cell death by autophagy: facts and apparent artefacts. *Cell Death Differ* 19: 87-95.
- DENTON, D., SHRAVAGE, B., SIMIN, R., MILLS, K., BERRY, D.L., BAEHRECKE, E.H. and KUMAR, S. (2009). Autophagy, not apoptosis, is essential for midgut cell death in *Drosophila*. *Curr Biol* 19: 1741-1746.
- DIETER, S.M., BALL, C.R., HOFFMANN, C.M., NOWROUZI, A., HERBST, F., ZAVIDIJ, O., ABEL, U., ARENS, A., WEICHERT, W., BRAND, K. *et al.*, (2011). Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. *Cell Stem Cell* 9: 357-365.
- DING, Z.B., SHI, Y.H., ZHOU, J., QIU, S.J., XU, Y., DAI, Z., SHI, G.M., WANG, X.Y., KE, A.W., WU, B. *et al.*, (2008). Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma. *Cancer Res* 68: 9167-9175.
- DRIESSENS, G., BECK, B., CAAUWE, A., SIMONS, B.D. and BLANPAIN, C. (2012). Defining the mode of tumour growth by clonal analysis. *Nature* 488: 527-530.
- DUBROVSKA, A., KIM, S., SALAMONE, R.J., WALKER, J.R., MAIRA, S.M., GARCIA-CHEVERRIA, C., SCHULTZ, P.G. and REDDY, V.A. (2009). The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. *Proc Natl Acad Sci USA* 106: 268-273.
- EGAN, D.F., SHACKELFORD, D.B., MIHAYLOVA, M.M., GELINO, S., KOHNZ, R.A., MAIR, W., VASQUEZ, D.S., JOSHI, A., GWINN, D.M., TAYLOR, R. *et al.*, (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331: 456-461.
- ELZINGA, B.M., NYHAN, M.J., CROWLEY, L.C., O'DONOVAN, T.R., CAHILL, M.R. and MCKENNA, S.L. (2013). Induction of autophagy by Imatinib sequesters Bcr-Abl in autophagosomes and down-regulates Bcr-Abl protein. *Am J Hematol* 88: 455-462.
- ERAMO, A., LOTTI, F., SETTE, G., PILOZZI, E., BIFFONI, M., DI VIRGILIO, A., CONTICELLO, C., RUCO, L., PESCHLE, C. and DE MARIA, R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 15: 504-514.
- ERAMO, A., RICCI-VITIANI, L., ZEUNER, A., PALLINI, R., LOTTI, F., SETTE, G., PILOZZI, E., LAROCCA, L.M., PESCHLE, C. and DE MARIA, R. (2006). Chemotherapy resistance of glioblastoma stem cells. *Cell Death Differ* 13: 1238-1241.
- ERDELYI, P., BORSOS, E., TAKACS-VELLAI, K., KOVACS, T., KOVACS, A.L., SIGMOND, T., HARGITAI, B., PASZTOR, L., SENGUPTA, T., DENG, M. *et al.*, (2011). Shared developmental roles and transcriptional control of autophagy and apoptosis in *Caenorhabditis elegans*. *J Cell Sci* 124: 1510-1518.
- ESPINA, V., MARIANI, B.D., GALLAGHER, R.I., TRAN, K., BANKS, S., WIEDEMANN, J., HURYK, H., MUELLER, C., ADAMO, L., DENG, J. *et al.*, (2010). Malignant precursor cells pre-exist in human breast DCIS and require autophagy for survival. *PLoS One* 5: e10240.
- FADER, C.M., SANCHEZ, D.G., MESTRE, M.B. and COLOMBO, M.I. (2009). TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. *Biochim Biophys Acta* 1793: 1901-1916.
- FILIPPI-CHIELA, E.C., VILLODRE, E.S., ZAMIN, L.L. and LENZ, G. (2011). Autophagy interplay with apoptosis and cell cycle regulation in the growth inhibiting effect of resveratrol in glioma cells. *PLoS One* 6: e20849.
- FIRAT, E., WEYERBROCK, A., GAEDICKE, S., GROSU, A.L. and NIEDERMANN, G. (2012). Chloroquine or chloroquine-PI3K/Akt pathway inhibitor combinations strongly promote gamma-irradiation-induced cell death in primary stem-like glioma cells. *PLoS One* 7: e47357.
- FRANCESCANGELI, F., PATRIZI, M., SIGNORE, M., FEDERICI, G., DI FRANCO, S., PAGLIUCA, A., BAIOCCHI, M., BIFFONI, M., RICCI VITIANI, L., TODARO, M. *et al.*, (2012). Proliferation state and polo-like kinase1 dependence of tumorigenic colon cancer cells. *Stem Cells* 30: 1819-1830.
- FRANCIPANE, M.G. and LAGASSE, E. (2013). Selective targeting of human colon cancer stem-like cells by the mTOR inhibitor Torin-1. *Oncotarget* 4: 1948-1962.
- FU, J., LIU, Z.G., LIU, X.M., CHEN, F.R., SHI, H.L., PANGJESSE, C.S., NG, H.K. and CHEN, Z.P. (2009). Glioblastoma stem cells resistant to temozolomide-induced autophagy. *Chin Med J (Engl)* 122: 1255-1259.
- FU, Y., CHANG, H., PENG, X., BAI, Q., YI, L., ZHOU, Y., ZHU, J. and MI, M. (2014). Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/beta-catenin signaling pathway. *PLoS One* 9: e102535.
- FUNDERBURK, S.F., WANG, Q.J. and YUE, Z. (2010). The Beclin 1-VPS34 complex--at the crossroads of autophagy and beyond. *Trends Cell Biol* 20: 355-362.
- FURUYA, N., YU, J., BYFIELD, M., PATTINGRE, S. and LEVINE, B. (2005). The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. *Autophagy* 1: 46-52.
- GALAVOTTI, S., BARTESAGHI, S., FACCENDA, D., SHAKED-RABI, M., SANZONE, S., MCEVOY, A., DINSDALE, D., CONDORELLI, F., BRANDNER, S., CAMPANELLA, M. *et al.*, (2013). The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. *Oncogene* 32: 699-712.
- GALLUZZI, L., BRAVO-SAN PEDRO, J.M., VITALE, I., AARONSON, S.A., ABRAMS, J.M., ADAM, D., ALNEMRI, E.S., ALTUCCI, L., ANDREWS, D., ANNICCHIARICO-PETRUZZELLI, M. *et al.*, (2015). Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. *Cell Death Differ.* 22: 58-73.
- GALLUZZI, L., KEPP, O., VANDER HEIDEN, M.G. and KROEMER, G. (2013). Metabolic targets for cancer therapy. *Nat Rev Drug Discov* 12: 829-846.
- GALLUZZI, L., PIETROCOLA, F., LEVINE, B. and KROEMER, G. (2014). Metabolic Control of Autophagy. *Cell* 159: 1263-1276.
- GALLUZZI, L., VITALE, I., ABRAMS, J.M., ALNEMRI, E.S., BAEHRECKE, E.H., BLAGOSKLONNY, M.V., DAWSON, T.M., DAWSON, V.L., EL-DEIRY, W.S., FULDA, S. *et al.*, (2012). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ* 19: 107-120.
- GANLEY, I.G., LAM DU, H., WANG, J., DING, X., CHEN, S. and JIANG, X. (2009). ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* 284: 12297-12305.
- GARNER, J.M., FAN, M., YANG, C.H., DU, Z., SIMS, M., DAVIDOFF, A.M. and PFEFFER, L.M. (2013). Constitutive activation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappaB signaling in glioblastoma cancer stem cells regulates the Notch pathway. *J Biol Chem* 288: 26167-26176.
- GERLINGER, M., ROWAN, A.J., HORSWELL, S., LARKIN, J., ENDEFELDER, D., GRONROOS, E., MARTINEZ, P., MATTHEWS, N., STEWART, A., TARPEY, P. *et al.*, (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 366: 883-892.
- GIANNONI, E., BIANCHINI, F., MASIERI, L., SERNI, S., TORRE, E., CALORINI, L. and CHIARUGI, P. (2010). Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. *Cancer Res* 70: 6945-6956.
- GINESTIER, C., HUR, M.H., CHARAFE-JAUFFRET, E., MONVILLE, F., DUTCHER, J., BROWN, M., JACQUEMIER, J., VIENS, P., KLEER, C.G., LIU, S. *et al.*, (2007). ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 1: 555-567.
- GOLEBIEWSKA, A., BRONS, N.H., BJERKVIG, R. and NICLOU, S.P. (2011). Critical appraisal of the side population assay in stem cell and cancer stem cell research. *Cell Stem Cell* 8: 136-147.
- GONG, C., BAUVY, C., TONELLI, G., YUE, W., DELOMENE, C., NICOLAS, V., ZHU,

- Y., DOMERGUE, V., MARIN-ESTEBAN, V., THARINGER, H. *et al.*, (2013). Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* 32: 2261-2272.
- GORDON, D.J., RESIO, B. and PELLMAN, D. (2012). Causes and consequences of aneuploidy in cancer. *Nat Rev Genet* 13: 189-203.
- GRANDER, D., KHARAZIHA, P., LAANE, E., POKROVSKAJA, K. and PANARETAKIS, T. (2009). Autophagy as the main means of cytotoxicity by glucocorticoids in hematological malignancies. *Autophagy* 5: 1198-1200.
- GREAVES, M. (2013). Cancer stem cells as 'units of selection'. *Evol Appl* 6: 102-108.
- GREEN, D.R. and LEVINE, B. (2014). To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* 157: 65-75.
- GRIGUER, C.E., OLIVA, C.R., GOBIN, E., MARCORELLES, P., BENOS, D.J., LANCASTER, J.R., JR. and GILLESPIE, G.Y. (2008). CD133 is a marker of bioenergetic stress in human glioma. *PLoS One* 3: e3655.
- GUAN, J.L., SIMON, A.K., PRESCOTT, M., MENENDEZ, J.A., LIU, F., WANG, F., WANG, C., WOLVETANG, E., VAZQUEZ-MARTIN, A. and ZHANG, J. (2013). Autophagy in stem cells. *Autophagy* 9: 830-849.
- GUO, J.Y., CHEN, H.Y., MATHEW, R., FAN, J., STROHECKER, A.M., KARSLI-UZUNBAS, G., KAMPHORST, J.J., CHEN, G., LEMONS, J.M., KARANTZA, V. *et al.*, (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* 25: 460-470.
- GUO, J.Y., KARSLI-UZUNBAS, G., MATHEW, R., AISNER, S.C., KAMPHORST, J.J., STROHECKER, A.M., CHEN, G., PRICE, S., LU, W., TENG, X. *et al.*, (2013). Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev* 27: 1447-1461.
- GUPTA, P.B., FILLMORE, C.M., JIANG, G., SHAPIRA, S.D., TAO, K., KUPERWASSER, C. and LANDER, E.S. (2011). Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* 146: 633-644.
- GUPTA, P.B., ONDER, T.T., JIANG, G., TAO, K., KUPERWASSER, C., WEINBERG, R.A. and LANDER, E.S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 138: 645-659.
- GWINN, D.M., SHACKELFORD, D.B., EGAN, D.F., MIHAYLOVA, M.M., MERY, A., VASQUEZ, D.S., TURK, B.E. and SHAW, R.J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 30: 214-226.
- HAMASAKI, M., FURUTA, N., MATSUDA, A., NEZU, A., YAMAMOTO, A., FUJITA, N., OOMORI, H., NODA, T., HARAGUCHI, T., HIRAOKA, Y. *et al.*, (2013). Autophagosomes form at ER-mitochondria contact sites. *Nature* 495: 389-393.
- HANAHAN, D. and COUSSENS, L.M. (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 21: 309-322.
- HANAHAN, D. and WEINBERG, R.A. (2011). Hallmarks of cancer: the next generation. *Cell* 144: 646-674.
- HARDIE, D.G., ROSS, F.A. and HAWLEY, S.A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 13: 251-262.
- HAYASHI-NISHINO, M., FUJITA, N., NODA, T., YAMAGUCHI, A., YOSHIMORI, T. and YAMAMOTO, A. (2009). A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* 11: 1433-1437.
- HEDDLESTON, J.M., LI, Z., MCLENDON, R.E., HJELMELAND, A.B. and RICH, J.N. (2009). The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* 8: 3274-3284.
- HEMMATI, H.D., NAKANO, I., LAZAREFF, J.A., MASTERMAN-SMITH, M., GESCHWIND, D.H., BRONNER-FRASER, M. and KORNBLUM, H.I. (2003). Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci USA* 100: 15178-15183.
- HERMANN, P.C., HUBER, S.L., HERRLER, T., AICHER, A., ELLWART, J.W., GUBA, M., BRUNS, C.J. and HEESCHEN, C. (2007). Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 1: 313-323.
- HIRSCH, H.A., ILIOPOULOS, D., TSICHLIS, P.N. and STRUHL, K. (2009). Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* 69: 7507-7511.
- HOSOKAWA, N., HARA, T., KAIZUKA, T., KISHI, C., TAKAMURA, A., MIURA, Y., IEMURA, S., NATSUME, T., TAKEHANA, K., YAMADA, N. *et al.*, (2009). Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 20: 1981-1991.
- HUANG, J.J., ZHU, Y.J., LIN, T.Y., JIANG, W.Q., HUANG, H.Q. and LI, Z.M. (2011). Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Hum Pathol* 42: 1459-1466.
- HUO, Y., CAI, H., TEPLOVA, I., BOWMAN-COLIN, C., CHEN, G., PRICE, S., BARNARD, N., GANESAN, S., KARANTZA, V., WHITE, E. *et al.*, (2013). Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. *Cancer Discov* 3: 894-907.
- IANNELLO, A. and RAULET, D.H. (2014). Immunosurveillance of senescent cancer cells by natural killer cells. *Oncoimmunology* 3: e27616.
- IANNELLO, A., THOMPSON, T.W., ARDOLINO, M., LOWE, S.W. and RAULET, D.H. (2013). p53-dependent chemokine production by senescent tumor cells supports NKG2D-dependent tumor elimination by natural killer cells. *J Exp Med* 210: 2057-2069.
- ILIOPOULOS, D., HIRSCH, H.A., WANG, G. and STRUHL, K. (2011). Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci USA* 108: 1397-1402.
- INAMI, Y., WAGURI, S., SAKAMOTO, A., KOUNO, T., NAKADA, K., HINO, O., WATANABE, S., ANDO, J., IWADATE, M., YAMAMOTO, M. *et al.*, (2011). Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol* 193: 275-284.
- INOKI, K., ZHU, T. and GUAN, K.L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115: 577-590.
- ISHIZAWA, K., RASHEED, Z.A., KARISCH, R., WANG, Q., KOWALSKI, J., SUSKY, E., PEREIRA, K., KARAMBOULAS, C., MOGHAL, N., RAJESHKUMAR, N.V. *et al.*, (2010). Tumor-initiating cells are rare in many human tumors. *Cell Stem Cell* 7: 279-282.
- JAIN, A., LAMARK, T., SJOTTEM, E., LARSEN, K.B., AWUH, J.A., OVERVATN, A., MCMAHON, M., HAYES, J.D. and JOHANSEN, T. (2010). p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* 285: 22576-22591.
- JIANG, H., GOMEZ-MANZANO, C., AOKI, H., ALONSO, M.M., KONDO, S., MCCORMICK, F., XU, J., KONDO, Y., BEKELE, B.N., COLMAN, H. *et al.*, (2007). Examination of the therapeutic potential of Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. *J Natl Cancer Inst* 99: 1410-1414.
- JIANG, Z.F., SHAO, L.J., WANG, W.M., YAN, X.B. and LIU, R.Y. (2012). Decreased expression of Beclin-1 and LC3 in human lung cancer. *Mol Biol Rep* 39: 259-267.
- JOHANSEN, T. and LAMARK, T. (2011). Selective autophagy mediated by autophagic adapter proteins. *Autophagy* 7: 279-296.
- JOPLING, C., BOUE, S. and IZPISUA BELMONTE, J.C. (2011). Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat Rev Mol Cell Biol* 12: 79-89.
- JUNG, C.H., JUN, C.B., RO, S.H., KIM, Y.M., OTTO, N.M., CAO, J., KUNDU, M. and KIM, D.H. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 20: 1992-2003.
- KANG, M.R., KIM, M.S., OH, J.E., KIM, Y.R., SONG, S.Y., KIM, S.S., AHN, C.H., YOO, N.J. and LEE, S.H. (2009). Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. *J Pathol* 217: 702-706.
- KANTARA, C., O'CONNELL, M., SARKAR, S., MOYA, S., ULLRICH, R. and SINGH, P. (2014). Curcumin promotes autophagic survival of a subset of colon cancer stem cells, which are ablated by DCLK1-siRNA. *Cancer Res* 74: 2487-2498.
- KARANTZA-WADSWORTH, V., PATEL, S., KRAVCHUK, O., CHEN, G., MATHEW, R., JIN, S. and WHITE, E. (2007). Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev* 21: 1621-1635.
- KASTAN, M.B., SCHLAFFER, E., RUSSO, J.E., COLVIN, O.M., CIVIN, C.I. and HILTON, J. (1990). Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood* 75: 1947-1950.
- KENDZIORRA, E., AHLBORN, K., SPITZNER, M., RAVE-FRANK, M., EMONS, G., GAEDCKE, J., KRAMER, F., WOLFF, H.A., BECKER, H., BEISSBARTH, T. *et al.*, (2011). Silencing of the Wnt transcription factor TCF4 sensitizes colorectal cancer cells to (chemo-) radiotherapy. *Carcinogenesis* 32: 1824-1831.
- KIM, H.S., LEE, S.H., DO, S.I., LIM, S.J., PARK, Y.K. and KIM, Y.W. (2011a). Clinicopathologic correlation of beclin-1 expression in pancreatic ductal adenocarcinoma.

- Pathol Res Pract* 207: 247-252.
- KIM, J., KIM, Y.C., FANG, C., RUSSELL, R.C., KIM, J.H., FAN, W., LIU, R., ZHONG, Q. and GUAN, K.L. (2013). Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* 152: 290-303.
- KIM, J., KUNDU, M., VIOLLET, B. and GUAN, K.L. (2011b). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 13: 132-141.
- KIM, M.S., SONG, S.Y., LEE, J.Y., YOO, N.J. and LEE, S.H. (2011c). Expression and mutational analyses of ATG5 gene in prostate cancers. *APMIS* 119: 802-807.
- KIMMELMAN, A.C. (2011). The dynamic nature of autophagy in cancer. *Genes Dev* 25: 1999-2010.
- KONG, Y., YOSHIDA, S., SAITO, Y., DOI, T., NAGATOSHI, Y., FUKATA, M., SAITO, N., YANG, S.M., IWAMOTO, C., OKAMURA, J. *et al.*, (2008). CD34+CD38+CD19+ as well as CD34+CD38-CD19+ cells are leukemia-initiating cells with self-renewal capacity in human B-precursor ALL. *Leukemia* 22: 1207-1213.
- KRESO, A. and DICK, J.E. (2014). Evolution of the cancer stem cell model. *Cell Stem Cell* 14: 275-291.
- KRESO, A., O'BRIEN, C.A., VAN GALEN, P., GAN, O.I., NOTTA, F., BROWN, A.M., NG, K., MA, J., WIENHOLDS, E., DUNANT, C. *et al.*, (2013). Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science* 339: 543-548.
- KROEMER, G., MARINO, G. and LEVINE, B. (2010). Autophagy and the integrated stress response. *Mol Cell* 40: 280-293.
- KUMA, A. and MIZUSHIMA, N. (2010). Physiological role of autophagy as an intracellular recycling system: with an emphasis on nutrient metabolism. *Semin Cell Dev Biol* 21: 683-690.
- KUMAR, D., SHANKAR, S. and SRIVASTAVA, R.K. (2013). Rottlerin-induced autophagy leads to the apoptosis in breast cancer stem cells: molecular mechanisms. *Mol Cancer* 12: 171.
- KUMAR, D., SHANKAR, S. and SRIVASTAVA, R.K. (2014). Rottlerin induces autophagy and apoptosis in prostate cancer stem cells via PI3K/Akt/mTOR signaling pathway. *Cancer Lett* 343: 179-189.
- LAANE, E., TAMM, K.P., BUENTKE, E., ITO, K., KHARAZIHA, P., OSCARSSON, J., CORCORAN, M., BJORKLUND, A.C., HULTENBY, K., LUNDIN, J. *et al.*, (2009). Cell death induced by dexamethasone in lymphoid leukemia is mediated through initiation of autophagy. *Cell Death Differ* 16: 1018-1029.
- LADDHA, S.V., GANESAN, S., CHAN, C.S. and WHITE, E. (2014). Mutational landscape of the essential autophagy gene BECN1 in human cancers. *Mol Cancer Res* 12: 485-490.
- LAMB, C.A., YOSHIMORI, T. and TOOZE, S.A. (2013). The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 14: 759-774.
- LAMY, L., NGO, V.N., EMRE, N.C., SHAFFER, A.L., 3RD, YANG, Y., TIAN, E., NAIR, V., KRULAK, M.J., ZINGONE, A., LANDGREN, O. *et al.*, (2013). Control of autophagic cell death by caspase-10 in multiple myeloma. *Cancer Cell* 23: 435-449.
- LAPIDOT, T., SIRARD, C., VORMOOR, J., MURDOCH, B., HOANG, T., CACERES-CORTES, J., MINDEN, M., PATERSON, B., CALIGIURI, M.A. and DICK, J.E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 367: 645-648.
- LAPLANTE, M. and SABATINI, D.M. (2012). mTOR signaling in growth control and disease. *Cell* 149: 274-293.
- LAZOVA, R., CAMP, R.L., KLUMP, V., SIDDIQUI, S.F., AMARAVADI, R.K. and PAWELEK, J.M. (2012). Punctate LC3B expression is a common feature of solid tumors and associated with proliferation, metastasis, and poor outcome. *Clin Cancer Res* 18: 370-379.
- LEVINE, B. and KROEMER, G. (2008). Autophagy in the pathogenesis of disease. *Cell* 132: 27-42.
- LI, B.X., LI, C.Y., PENG, R.Q., WU, X.J., WANG, H.Y., WAN, D.S., ZHU, X.F. and ZHANG, X.S. (2009a). The expression of beclin 1 is associated with favorable prognosis in stage IIIB colon cancers. *Autophagy* 5: 303-306.
- LI, C., LEE, C.J. and SIMEONE, D.M. (2009b). Identification of human pancreatic cancer stem cells. *Methods Mol Biol* 568: 161-173.
- LIANG, X.H., JACKSON, S., SEAMAN, M., BROWN, K., KEMPKE, B., HIBSHOOSH, H. and LEVINE, B. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402: 672-676.
- LIN, Q. and YUN, Z. (2010). Impact of the hypoxic tumor microenvironment on the regulation of cancer stem cell characteristics. *Cancer Biol Ther* 9: 949-956.
- LIN, Y.H., HUANG, Y.C., CHEN, L.H. and CHU, P.M. (2015). Autophagy in cancer stem/progenitor cells. *Cancer Chemother Pharmacol* 75: 879-886.
- LIU, H., HE, Z., VON RUTTE, T., YOUSEFI, S., HUNGER, R.E. and SIMON, H.U. (2013). Down-regulation of autophagy-related protein 5 (ATG5) contributes to the pathogenesis of early-stage cutaneous melanoma. *Sci Transl Med* 5: 202ra123.
- LOMONACO, S.L., FINNISS, S., XIANG, C., DECARVALHO, A., UMANSKY, F., KALKANIS, S.N., MIKKELSEN, T. and BRODIE, C. (2009). The induction of autophagy by gamma-radiation contributes to the radioresistance of glioma stem cells. *Int J Cancer* 125: 717-722.
- MA, Y., LIANG, D., LIU, J., AXCRONA, K., KVALHEIM, G., STOKKE, T., NESLAND, J.M. and SUO, Z. (2011). Prostate cancer cell lines under hypoxia exhibit greater stem-like properties. *PLoS One* 6: e29170.
- MAGEE, J.A., PISKOUNOVA, E. and MORRISON, S.J. (2012). Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 21: 283-296.
- MAI, T.T., MOON, J., SONG, Y., VIET, P.Q., PHUC, P.V., LEE, J.M., YI, T.H., CHO, M. and CHO, S.K. (2012). Ginsenoside F2 induces apoptosis accompanied by protective autophagy in breast cancer stem cells. *Cancer Lett* 321: 144-153.
- MAJETI, R., CHAO, M.P., ALIZADEH, A.A., PANG, W.W., JAISWAL, S., GIBBS, K.D., JR., VAN ROOIJEN, N. and WEISSMAN, I.L. (2009). CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 138: 286-299.
- MANIC, G., OBRIST, F., KROEMER, G., VITALE, I. and GALLUZZI, L. (2014). Chloroquine and hydroxychloroquine for cancer therapy. *Molec. Cell. Oncol.* 1: e29911.
- MARI, M., GRIFFITH, J., RIETER, E., KRISHNAPPA, L., KLIONSKY, D.J. and REGGIORI, F. (2010). An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. *J Cell Biol* 190: 1005-1022.
- MARINO, G., NISO-SANTANO, M., BAEHRECKE, E.H. and KROEMER, G. (2014). Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol* 15: 81-94.
- MARINO, G., SALVADOR-MONTOLIU, N., FUEYO, A., KNECHT, E., MIZUSHIMA, N. and LOPEZ-OTIN, C. (2007). Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem* 282: 18573-18583.
- MARTELLI, A.M., EVANGELISTI, C., FOLLO, M.Y., RAMAZZOTTI, G., FINI, M., GIARDINO, R., MANZOLI, L., MCCUBREY, J.A. and COCCO, L. (2011). Targeting the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in cancer stem cells. *Curr Med Chem* 18: 2715-2726.
- MATHEW, R., KONGARA, S., BEAUDOIN, B., KARP, C.M., BRAY, K., DEGENHARDT, K., CHEN, G., JIN, S. and WHITE, E. (2007). Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev* 21: 1367-1381.
- MATHIEU, J., ZHANG, Z., ZHOU, W., WANG, A.J., HEDDLESTON, J.M., PINNA, C.M., HUBAUD, A., STADLER, B., CHOI, M., BAR, M. *et al.*, (2011). HIF induces human embryonic stem cell markers in cancer cells. *Cancer Res* 71: 4640-4652.
- MAUGERI-SACCA, M., VIGNERI, P. and DE MARIA, R. (2011). Cancer stem cells and chemosensitivity. *Clin Cancer Res* 17: 4942-4947.
- MAYCOTTE, P., ARYAL, S., CUMMINGS, C.T., THORBURN, J., MORGAN, M.J. and THORBURN, A. (2012). Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. *Autophagy* 8: 200-2012.
- MCAULIFFE, S.M., MORGAN, S.L., WYANT, G.A., TRAN, L.T., MUTO, K.W., CHEN, Y.S., CHIN, K.T., PARTRIDGE, J.C., POOLE, B.B., CHENG, K.H. *et al.*, (2012). Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy. *Proc Natl Acad Sci USA* 109: E2939-E2948.
- MEACHAM, C.E. and MORRISON, S.J. (2013). Tumour heterogeneity and cancer cell plasticity. *Nature* 501: 328-337.
- MEIJER, A.J. and KLIONSKY, D.J. (2011). Vps34 is a phosphatidylinositol 3-kinase, not a phosphoinositide 3-kinase. *Autophagy* 7: 563-564.
- MICHAUD, M., MARTINS, I., SUKKURWALA, A.Q., ADJEMIAN, S., MA, Y., PELLEGATTI, P., SHEN, S., KEPP, O., SCOAZEC, M., MIGNOT, G. *et al.*, (2011). Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* 334: 1573-1577.
- MICHELS, J., OBRIST, F., CASTEDO, M., VITALE, I. and KROEMER, G. (2015). PARP and other prospective targets for poisoning cancer cell metabolism. *Biochem Pharmacol* 92: 164-171.
- MIZUSHIMA, N. (2010). The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell Biol* 22: 132-139.

- MIZUSHIMA, N. and KOMATSU, M. (2011). Autophagy: renovation of cells and tissues. *Cell* 147: 728-741.
- MIZUSHIMA, N., LEVINE, B., CUERVO, A.M. and KLIONSKY, D.J. (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069-1075.
- MIZUSHIMA, N., YOSHIMORI, T. and OHSUMI, Y. (2011). The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 27: 107-132.
- MOHAMMED, A., JANAKIRAM, N.B., BREWER, M., RITCHIE, R.L., MARYA, A., LIGHTFOOT, S., STEELE, V.E. and RAO, C.V. (2013). Antidiabetic Drug Metformin Prevents Progression of Pancreatic Cancer by Targeting in Part Cancer Stem Cells and mTOR Signaling. *Transl Oncol* 6: 649-659.
- MOHYELDIN, A., GARZON-MUVDI, T. and QUINONES-HINOJOSA, A. (2010). Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 7: 150-161.
- MORSELLI, E., GALLUZZI, L., KEPP, O., MARINO, G., MICHAUD, M., VITALE, I., MAIURI, M.C. and KROEMER, G. (2011). Oncosuppressive functions of autophagy. *Antioxid Redox Signal* 14: 2251-2269.
- NAIR, U., JOTWANI, A., GENG, J., GAMMOH, N., RICHERSON, D., YEN, W.L., GRIFFITH, J., NAG, S., WANG, K., MOSS, T. et al., (2011). SNARE proteins are required for macroautophagy. *Cell* 146: 290-302.
- NAKATOGAWA, H., ICHIMURA, Y. and OHSUMI, Y. (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130: 165-178.
- NEZIS, I.P., SHRAVAGE, B.V., SAGONA, A.P., LAMARK, T., BJORKOY, G., JOHANSEN, T., RUSTEN, T.E., BRECH, A., BAEHRECKE, E.H. and STENMARK, H. (2010). Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late *Drosophila melanogaster* oogenesis. *J Cell Biol* 190: 523-531.
- NGUYEN, L.V., VANNER, R., DIRKS, P. and EAVES, C.J. (2012). Cancer stem cells: an evolving concept. *Nat Rev Cancer* 12: 133-143.
- NIXON, R.A. (2013). The role of autophagy in neurodegenerative disease. *Nat Med* 19: 983-997.
- NODA, T., FUJITA, N. and YOSHIMORI, T. (2008). The Ubi brothers reunited. *Autophagy* 4: 540-541.
- O'DONOVAN, T.R., O'SULLIVAN, G.C. and MCKENNA, S.L. (2011). Induction of autophagy by drug-resistant esophageal cancer cells promotes their survival and recovery following treatment with chemotherapeutics. *Autophagy* 7: 509-524.
- OHSUMI, Y. (2001). Molecular dissection of autophagy: two ubiquitin-like systems. *Nat Rev Mol Cell Biol* 2: 211-216.
- OJHA, R., JHA, V., SINGH, S.K. and BHATTACHARYYA, S. (2014). Autophagy inhibition suppresses the tumorigenic potential of cancer stem cell enriched side population in bladder cancer. *Biochim Biophys Acta* 1842: 2073-2086.
- PALLINI, R., RICCI-VITIANI, L., BANNA, G.L., SIGNORE, M., LOMBARDI, D., TODARO, M., STASSI, G., MARTINI, M., MAIRA, G., LAROCCA, L.M. et al., (2008). Cancer stem cell analysis and clinical outcome in patients with glioblastoma multiforme. *Clin Cancer Res* 14: 8205-8212.
- PARKHITKO, A., MYACHINA, F., MORRISON, T.A., HINDI, K.M., AURICCHIO, N., KARBOWNICZEK, M., WU, J.J., FINKEL, T., KWIATKOWSKI, D.J., YU, J.J. et al., (2011). Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. *Proc Natl Acad Sci USA* 108: 12455-12460.
- PATRAWALA, L., CALHOUN, T., SCHNEIDER-BROUSSARD, R., LI, H., BHATIA, B., TANG, S., REILLY, J.G., CHANDRA, D., ZHOU, J., CLAYPOOL, K. et al., (2006). Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 25: 1696-1708.
- PATTINGRE, S., TASSA, A., QU, X., GARUTI, R., LIANG, X.H., MIZUSHIMA, N., PACKER, M., SCHNEIDER, M.D. and LEVINE, B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122: 927-939.
- PECE, S., TOSONI, D., CONFALONIERI, S., MAZZAROL, G., VECCHI, M., RONZONI, S., BERNARD, L., VIALE, G., PELICCI, P.G. and DI FIORE, P.P. (2010). Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. *Cell* 140: 62-73.
- PHADWAL, K., WATSON, A.S. and SIMON, A.K. (2013). Tightrope act: autophagy in stem cell renewal, differentiation, proliferation, and aging. *Cell Mol Life Sci* 70: 89-103.
- PHILLIPS, T.M., MCBRIDE, W.H. and PAJONK, F. (2006). The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst* 98: 1777-1785.
- PIRTOLI, L., CEVENINI, G., TINI, P., VANNINI, M., OLIVERI, G., MARSILI, S., MOURMOURAS, V., RUBINO, G. and MIRACCO, C. (2009). The prognostic role of Beclin 1 protein expression in high-grade gliomas. *Autophagy* 5: 930-936.
- POLSON, H.E., DE LARTIGUE, J., RIGDEN, D.J., REEDIJK, M., URBE, S., CLAGUE, M.J. and TOOZE, S.A. (2010). Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* 6: 506-522.
- QIANG, L., WU, T., ZHANG, H.W., LU, N., HU, R., WANG, Y.J., ZHAO, L., CHEN, F.H., WANG, X.T., YOU, Q.D. et al., (2012). HIF-1alpha is critical for hypoxia-mediated maintenance of glioblastoma stem cells by activating Notch signaling pathway. *Cell Death Differ* 19: 284-294.
- QU, X., YU, J., BHAGAT, G., FURUYA, N., HIBSHOOSH, H., TROXEL, A., ROSEN, J., ESKELINEN, E.L., MIZUSHIMA, N., OHSUMI, Y. et al., (2003). Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* 112: 1809-1820.
- QUINTANA, E., SHACKLETON, M., FOSTER, H.R., FULLEN, D.R., SABEL, M.S., JOHNSON, T.M. and MORRISON, S.J. (2010). Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell* 18: 510-523.
- QUINTANA, E., SHACKLETON, M., SABEL, M.S., FULLEN, D.R., JOHNSON, T.M. and MORRISON, S.J. (2008). Efficient tumour formation by single human melanoma cells. *Nature* 456: 593-598.
- RAO, R., BALUSU, R., FISKUS, W., MUDUNURU, U., VENKANNAGARI, S., CHAUHAN, L., SMITH, J.E., HEMBRUFF, S.L., HA, K., ATADJA, P. et al., (2012). Combination of pan-histone deacetylase inhibitor and autophagy inhibitor exerts superior efficacy against triple-negative human breast cancer cells. *Mol Cancer Ther* 11: 973-983.
- RAO, S., TORTOLA, L., PERLOT, T., WIRNSBERGER, G., NOVATSKOVA, M., NITSCH, R., SYKACEK, P., FRANK, L., SCHRAMEK, D., KOMNENOVIC, V. et al., (2014). A dual role for autophagy in a murine model of lung cancer. *Nat Commun* 5: 3056.
- RAUSCH, V., LIU, L., APEL, A., RETTIG, T., GLADKICH, J., LABSCH, S., KAL-LIFATIDIS, G., KACZOROWSKI, A., GROTH, A., GROSS, W. et al., (2012). Autophagy mediates survival of pancreatic tumour-initiating cells in a hypoxic microenvironment. *J Pathol* 227: 325-335.
- RAVIKUMAR, B., MOREAU, K., JAHREISS, L., PURI, C. and RUBINSZTEIN, D.C. (2010). Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 12: 747-757.
- RELLO-VARONA, S., LISSA, D., SHEN, S., NISO-SANTANO, M., SENOVILLA, L., MARINO, G., VITALE, I., JEMAA, M., HARPER, F., PIERRON, G. et al., (2012). Autophagic removal of micronuclei. *Cell Cycle* 11: 170-176.
- REYA, T., MORRISON, S.J., CLARKE, M.F. and WEISSMAN, I.L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* 414: 105-11.
- RICCI-VITIANI, L., LOMBARDI, D.G., PILOZZI, E., BIFFONI, M., TODARO, M., PESCHLE, C. and DE MARIA, R. (2007). Identification and expansion of human colon-cancer-initiating cells. *Nature* 445: 111-5.
- RICCI-VITIANI, L., PALLINI, R., LAROCCA, L.M., LOMBARDI, D.G., SIGNORE, M., PIERCONTI, F., PETRUCCI, G., MONTANO, N., MAIRA, G. and DE MARIA, R. (2008). Mesenchymal differentiation of glioblastoma stem cells. *Cell Death Differ* 15: 1491-8.
- ROESCH, A., FUKUNAGA-KALABIS, M., SCHMIDT, E.C., ZABIEROWSKI, S.E., BRAFFORD, P.A., VULTUR, A., BASU, D., GIMOTTY, P., VOGT, T. and HERLYN, M. (2010). A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* 141: 583-94.
- ROSENFELDT, M.T., O'PREY, J., MORTON, J.P., NIXON, C., MACKAY, G., MROWINSKA, A., AU, A., RAI, T.S., ZHENG, L., RIDGWAY, R. et al., (2013). p53 status determines the role of autophagy in pancreatic tumour development. *Nature* 504: 296-300.
- ROTHER, K., LIN, H., LIN, K.B., LEUNG, A., WANG, H.M., MALEKESMAEILI, M., BRINKMAN, R.R., FORREST, D.L., GORSKI, S.M. and JIANG, X. (2014). The core autophagy protein ATG4B is a potential biomarker and therapeutic target in CML stem/progenitor cells. *Blood* 123: 3622-34.
- RUBINSZTEIN, D.C., CODOGNO, P. and LEVINE, B. (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov* 11: 709-30.
- RUBINSZTEIN, D.C., MARINO, G. and KROEMER, G. (2011). Autophagy and aging. *Cell* 146: 682-95.
- RUSSELL, R.C., TIAN, Y., YUAN, H., PARK, H.W., CHANG, Y.Y., KIM, J., KIM, H.,

- NEUFELD, T.P., DILLIN, A. and GUAN, K.L. (2013). ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol* 15: 741-50.
- SASAKI, K., TSUNO, N.H., SUNAMI, E., TSURITA, G., KAWAI, K., OKAJI, Y., NISHIKAWA, T., SHUNO, Y., HONGO, K., HIYOSHI, M. *et al.*, (2010). Chloroquine potentiates the anti-cancer effect of 5-fluorouracil on colon cancer cells. *BMC Cancer* 10: 370.
- SCHEPERS, A.G., SNIPPET, H.J., STANGE, D.E., VAN DEN BORN, M., VAN ES, J.H., VAN DE WETERING, M. and CLEVERS, H. (2012). Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science* 337: 730-735.
- SCHULZE, A. and HARRIS, A.L. (2012). How cancer metabolism is tuned for proliferation and vulnerable to disruption. *Nature* 491: 364-373.
- SELVAKUMARAN, M., AMARAVADI, R.K., VASILEVSKAYA, I.A. and O'DWYER, P.J. (2013). Autophagy inhibition sensitizes colon cancer cells to antiangiogenic and cytotoxic therapy. *Clin Cancer Res* 19: 2995-3007.
- SHACKLETON, M., QUINTANA, E., FEARON, E.R. and MORRISON, S.J. (2009). Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* 138: 822-829.
- SHARMA, S.V., LEE, D.Y., LI, B., QUINLAN, M.P., TAKAHASHI, F., MAHESWARAN, S., MCDERMOTT, U., AZIZIAN, N., ZOU, L., FISCHBACH, M.A. *et al.*, (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 141: 69-80.
- SHRAVAGE, B.V., HILL, J.H., POWERS, C.M., WU, L. and BAEHRECKE, E.H. (2013). Atg6 is required for multiple vesicle trafficking pathways and hematopoiesis in *Drosophila*. *Development* 140: 1321-1329.
- SINGH, B.N., KUMAR, D., SHANKAR, S. and SRIVASTAVA, R.K. (2012). Rottlerin induces autophagy which leads to apoptotic cell death through inhibition of PI3K/Akt/mTOR pathway in human pancreatic cancer stem cells. *Biochem Pharmacol* 84: 1154-1163.
- SINGH, S.K., HAWKINS, C., CLARKE, I.D., SQUIRE, J.A., BAYANI, J., HIDE, T., HENKELMAN, R.M., CUSIMANO, M.D. and DIRKS, P.B. (2004). Identification of human brain tumour initiating cells. *Nature* 432: 396-401.
- SONG, Y.J., ZHANG, S.S., GUO, X.L., SUN, K., HAN, Z.P., LI, R., ZHAO, Q.D., DENG, W.J., XIE, X.Q., ZHANG, J.W. *et al.*, (2013). Autophagy contributes to the survival of CD133+ liver cancer stem cells in the hypoxic and nutrient-deprived tumor microenvironment. *Cancer Lett* 339: 70-81.
- SONG, Z., YUE, W., WEI, B., WANG, N., LI, T., GUAN, L., SHI, S., ZENG, Q., PEI, X. and CHEN, L. (2011). Sonic hedgehog pathway is essential for maintenance of cancer stem-like cells in human gastric cancer. *PLoS One* 6: e17687.
- SUN, L., MATHEWS, L.A., CABARCAS, S.M., ZHANG, X., YANG, A., ZHANG, Y., YOUNG, M.R., KLARMANN, K.D., KELLER, J.R. and FARRAR, W.L. (2013). Epigenetic regulation of SOX9 by the NF-kappaB signaling pathway in pancreatic cancer stem cells. *Stem Cells* 31: 1454-1466.
- SUVA, M.L., RIGGI, N. and BERNSTEIN, B.E. (2013). Epigenetic reprogramming in cancer. *Science* 339: 1567-1570.
- TAKAHASHI, Y., MEYERKORD, C.L., HORI, T., RUNKLE, K., FOX, T.E., KESTER, M., LOUGHRAN, T.P. and WANG, H.G. (2011). Bif-1 regulates Atg9 trafficking by mediating the fission of Golgi membranes during autophagy. *Autophagy* 7: 61-73.
- TAKAMURA, A., KOMATSU, M., HARA, T., SAKAMOTO, A., KISHI, C., WAGURI, S., EISHI, Y., HINO, O., TANAKA, K. and MIZUSHIMA, N. (2011). Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 25: 795-800.
- TAKEBE, N., HARRIS, P.J., WARREN, R.Q. and IVY, S.P. (2011). Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. *Nat Rev Clin Oncol* 8: 97-106.
- THUMM, M., EGNER, R., KOCH, B., SCHLUMBERGER, M., STRAUB, M., VEENHUIS, M. and WOLF, D.H. (1994). Isolation of autophagocytosis mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 349: 275-280.
- TODARO, M., ALEA, M.P., DI STEFANO, A.B., CAMMARERI, P., VERMEULEN, L., IOVINO, F., TRIPODO, C., RUSSO, A., GULOTTA, G., MEDEMA, J.P. *et al.*, (2007). Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 1: 389-402.
- TODARO, M., GAGGIANESI, M., CATALANO, V., BENFANTE, A., IOVINO, F., BIFFONI, M., APUZZO, T., SPERDUTI, I., VOLPE, S., COCORULLO, G. *et al.*, (2014). CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* 14: 342-356.
- TONG, Y., LIU, Y.Y., YOU, L.S. and QIAN, W.B. (2012). Perifosine induces protective autophagy and upregulation of ATG5 in human chronic myelogenous leukemia cells in vitro. *Acta Pharmacol Sin* 33: 542-550.
- TSUKADA, M. and OHSUMI, Y. (1993). Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 333: 169-174.
- ULASOV, I.V., NANDI, S., DEY, M., SONABEND, A.M. and LESNIAK, M.S. (2011). Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133(+) glioma stem cells to temozolomide therapy. *Mol Med* 17: 103-112.
- VANDERHEIDEN, M.G., CANTLEY, L.C. and THOMPSON, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324: 1029-1033.
- VERMEULEN, L., DE SOUSA E MELO, F., RICHEL, D.J. and MEDEMA, J.P. (2012). The developing cancer stem-cell model: clinical challenges and opportunities. *Lancet Oncol* 13: e83-e89.
- VERMEULEN, L., DE SOUSA, E.M.F., VAN DER HEIJDEN, M., CAMERON, K., DE JONG, J.H., BOROVSKI, T., TUYNMAN, J.B., TODARO, M., MERZ, C., RORDERMOND, H. *et al.*, (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 12: 468-476.
- VESSONI, A.T., MUOTRI, A.R. and OKAMOTO, O.K. (2012). Autophagy in stem cell maintenance and differentiation. *Stem Cells Dev* 21: 513-520.
- VIALE, A., PETTAZZONI, P., LYSSOTIS, C.A., YING, H., SANCHEZ, N., MARCHESINI, M., CARUGO, A., GREEN, T., SETH, S., GIULIANI, V. *et al.*, (2014). Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 514: 628-632.
- VITALE, I., GALLUZZI, L., CASTEDO, M. and KROEMER, G. (2011a). Mitotic catastrophe: a mechanism for avoiding genomic instability. *Nat Rev Mol Cell Biol* 12: 385-392.
- VITALE, I., GALLUZZI, L., SENOVILLA, L., CRIOLLO, A., JEMAA, M., CASTEDO, M. and KROEMER, G. (2011b). Illicit survival of cancer cells during polyploidization and depolyploidization. *Cell Death Differ* 18: 1403-1413.
- WAN, X.B., FAN, X.J., CHEN, M.Y., XIANG, J., HUANG, P.Y., GUO, L., WU, X.Y., XU, J., LONG, Z.J., ZHAO, Y. *et al.*, (2010). Elevated Beclin 1 expression is correlated with HIF-1alpha in predicting poor prognosis of nasopharyngeal carcinoma. *Autophagy* 6: 395-404.
- WANG, J., SAKARIASSEN, P.O., TSINKALOVSKY, O., IMMERSVOLL, H., BOE, S.O., SVENDSEN, A., PRESTEGARDEN, L., ROSLAND, G., THORSEN, F., STUHR, L. *et al.*, (2008). CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer* 122: 761-768.
- WANG, Y., WANG, X.D., LAPI, E., SULLIVAN, A., JIA, W., HE, Y.W., RATNAYAKA, I., ZHONG, S., GOLDIN, R.D., GOEMANS, C.G. *et al.*, (2012). Autophagic activity dictates the cellular response to oncogenic RAS. *Proc Natl Acad Sci USA* 109: 13325-13330.
- WEI, H., WEI, S., GAN, B., PENG, X., ZOU, W. and GUAN, J.L. (2011). Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes Dev* 25: 1510-1527.
- WEI, Y., JIANG, Y., ZOU, F., LIU, Y., WANG, S., XU, N., XU, W., CUI, C., XING, Y., CAO, B. *et al.*, (2013a). Activation of PI3K/Akt pathway by CD133-p85 interaction promotes tumorigenic capacity of glioma stem cells. *Proc Natl Acad Sci USA* 110: 6829-6834.
- WEI, Y., ZOU, Z., BECKER, N., ANDERSON, M., SUMPTER, R., XIAO, G., KINCH, L., KODURU, P., CHRISTUDASS, C.S., VELTRI, R.W. *et al.*, (2013b). EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance. *Cell* 154: 1269-1284.
- WEIDBERG, H., SHPILKA, T., SHVETS, E., ABADA, A., SHIMRON, F. and ELAZAR, Z. (2011). LC3 and GATE-16 N termini mediate membrane fusion processes required for autophagosome biogenesis. *Dev Cell* 20: 444-454.
- WEIDBERG, H., SHVETS, E., SHPILKA, T., SHIMRON, F., SHINDER, V. and ELAZAR, Z. (2010). LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. *EMBO J* 29: 1792-1802.
- WHITE, E. (2012). Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer* 12: 401-410.
- WIRTH, M., JOACHIM, J. and TOOZE, S.A. (2013). Autophagosome formation—the role of ULK1 and Beclin1-PI3KC3 complexes in setting the stage. *Semin Cancer Biol* 23: 301-309.
- WOLF, J., DEWI, D.L., FREDEBOHM, J., MULLER-DECKER, K., FLECHTENMACHER, C., HOHEISEL, J.D. and BOETTCHER, M. (2013). A mammosphere formation RNAi screen reveals that ATG4A promotes a breast cancer stem-like phenotype. *Breast Cancer Res* 15: R109.
- WU, S., WANG, X., CHEN, J. and CHEN, Y. (2013). Autophagy of cancer stem cells

- is involved with chemoresistance of colon cancer cells. *Biochem Biophys Res Commun* 434: 898-903.
- XIE, R., WANG, F., MCKEEHAN, W.L. and LIU, L. (2011). Autophagy enhanced by microtubule- and mitochondrion-associated MAP1S suppresses genome instability and hepatocarcinogenesis. *Cancer Res* 71: 7537-7546.
- XUE, W., ZENDER, L., MIETHING, C., DICKINS, R.A., HERNANDO, E., KRIZHANOVSKY, V., CORDON-CARDO, C. and LOWE, S.W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 445: 656-660.
- YANG, S., WANG, X., CONTINO, G., LIESA, M., SAHIN, E., YING, H., BAUSE, A., LI, Y., STOMMEL, J.M., DELL'ANTONIO, G. et al., (2011a). Pancreatic cancers require autophagy for tumor growth. *Genes Dev* 25: 717-729.
- YANG, Z. and KLIONSKY, D.J. (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 22: 124-131.
- YANG, Z., ZHANG, L., MA, A., LIU, L., LI, J., GU, J. and LIU, Y. (2011b). Transient mTOR inhibition facilitates continuous growth of liver tumors by modulating the maintenance of CD133+ cell populations. *PLoS One* 6: e28405.
- YENIGUN, V.B., OZPOLAT, B. and KOSE, G.T. (2013). Response of CD44+/CD24-/low breast cancer stem/progenitor cells to tamoxifen and doxorubicin-induced autophagy. *Int J Mol Med* 31: 1477-1483.
- YOULE, R.J. and NARENDRA, D.P. (2011). Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* 12: 9-14.
- YOUNG, A.R., NARITA, M., FERREIRA, M., KIRSCHNER, K., SADAIE, M., DARTOT, J.F., TAVARE, S., ARAKAWA, S., SHIMIZU, S., WATT, F.M. et al., (2009). Autophagy mediates the mitotic senescence transition. *Genes Dev* 23: 798-803.
- YU, Y., YANG, L., ZHAO, M., ZHU, S., KANG, R., VERNON, P., TANG, D. and CAO, L. (2012). Targeting microRNA-30a-mediated autophagy enhances imatinib activity against human chronic myeloid leukemia cells. *Leukemia* 26: 1752-1760.
- YUE, W., HAMAI, A., TONELLI, G., BAUVY, C., NICOLAS, V., THARINGER, H., CODOGNO, P. and MEHRPOUR, M. (2013). Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. *Autophagy* 9: 714-729.
- YUE, Z., JIN, S., YANG, C., LEVINE, A.J. and HEINTZ, N. (2003). Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA* 100: 15077-15082.
- ZENG, M. and ZHOU, J.N. (2008). Roles of autophagy and mTOR signaling in neuronal differentiation of mouse neuroblastoma cells. *Cell Signal* 20: 659-665.
- ZEUNER, A., FRANCESCANGELI, F., CONTAVALLI, P., ZAPPARELLI, G., APUZZO, T., ERAMO, A., BAIOCCHI, M., DE ANGELIS, M.L., BIFFONI, M., SETTE, G. et al., (2014a). Elimination of quiescent/slow-proliferating cancer stem cells by Bcl-X inhibition in non-small cell lung cancer. *Biochem Pharmacol* 92: 164-171.
- ZEUNER, A., TODARO, M., STASSI, G. and DE MARIA, R. (2014b). Colorectal Cancer Stem Cells: From the Crypt to the Clinic. *Cell Stem Cell* 15: 692-705.
- ZHAO, Y., HUANG, Q., YANG, J., LOU, M., WANG, A., DONG, J., QIN, Z. and ZHANG, T. (2010). Autophagy impairment inhibits differentiation of glioma stem/progenitor cells. *Brain Res* 1313: 250-258.
- ZHOU, J., WULFKUHL, J., ZHANG, H., GU, P., YANG, Y., DENG, J., MARGOLICK, J.B., LIOTTA, L.A., PETRICOIN, E., 3RD and ZHANG, Y. (2007). Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. *Proc Natl Acad Sci USA* 104: 16158-16163.
- ZHU, H., WANG, D., LIU, Y., SU, Z., ZHANG, L., CHEN, F., ZHOU, Y., WU, Y., YU, M., ZHANG, Z. et al., (2013). Role of the Hypoxia-inducible factor-1 alpha induced autophagy in the conversion of non-stem pancreatic cancer cells into CD133+ pancreatic cancer stem-like cells. *Cancer Cell Int* 13: 119.
- ZHU, H., WANG, D., ZHANG, L., XIE, X., WU, Y., LIU, Y., SHAO, G. and SU, Z. (2014). Upregulation of autophagy by hypoxia-inducible factor-1alpha promotes EMT and metastatic ability of CD133+ pancreatic cancer stem-like cells during intermittent hypoxia. *Oncol Rep* 32: 935-942.
- ZHUANG, W., LI, B., LONG, L., CHEN, L., HUANG, Q. and LIANG, Z. (2011a). Induction of autophagy promotes differentiation of glioma-initiating cells and their radiosensitivity. *Int J Cancer* 129: 2720-2731.
- ZHUANG, W., LI, B., LONG, L., CHEN, L., HUANG, Q. and LIANG, Z.Q. (2011b). Knockdown of the DNA-dependent protein kinase catalytic subunit radiosensitizes glioma-initiating cells by inducing autophagy. *Brain Res* 1371: 7-15.
- ZHUANG, W.Z., LONG, L.M., JI, W.J. and LIANG, Z.Q. (2011c). Rapamycin induces differentiation of glioma stem/progenitor cells by activating autophagy. *Chin J Cancer* 30: 712-720.

**Further Related Reading, published previously in the *Int. J. Dev. Biol.***

**Revisiting DNA damage repair, p53-mediated apoptosis and cisplatin sensitivity in germ cell tumors**

Francesca Cavallo, Darren R. Feldman and Marco Barchi  
*Int. J. Dev. Biol.* (2013) 57: 273-280

**Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development**

R. John Aitken and Mark A. Baker  
*Int. J. Dev. Biol.* (2013) 57: 265-272

**Regulation of programmed cell death during neural induction in the chick embryo**

Anna Gibson, Neil Robinson, Andrea Streit, Guojun Sheng and Claudio D. Stern  
*Int. J. Dev. Biol.* (2011) 55: 33-43

**Life-giving caspases: revealing new roles during mouse embryo preimplantation development**

Dolores Busso, Calixto Dominguez, Tomas Perez-Acle and Ricardo D. Moreno  
*Int. J. Dev. Biol.* (2010) 54: 857-865

**Both jnk and apoptosis pathways regulate growth and terminalia rotation during *Drosophila* genital disc development**

Sergio Benitez, Claudia Sosa, Nicolás Tomasini and Ana Macías  
*Int. J. Dev. Biol.* (2010) 54: 643-653

**Mpl receptor defect leads to earlier appearance of hematopoietic cells/hematopoietic stem cells in the Aorta-Gonad-Mesonephros region, with increased apoptosis**

Maud Fleury, Laurence Petit-Cocault, Denis Clay and Michèle Souyri  
*Int. J. Dev. Biol.* (2010) 54: 1067-1074

**Apoptosis in *Drosophila*: compensatory proliferation and undead cells**

Francisco A. Martín, Ainhoa Pérez-Garijo and Ginés Morata  
*Int. J. Dev. Biol.* (2009) 53: 1341-1347

**Key apoptosis regulating proteins are down-regulated during postnatal tissue development**

Shane D. Madden, Maryanne Donovan and Thomas G. Cotter  
*Int. J. Dev. Biol.* (2007) 51: 415-424

**NMDA-receptor blockade enhances cell apoptosis in the developing retina of the postnatal rat**

María Hernández, Inmaculada Guerrikagoitia, Luis Martínez-Millan and Elena Vecino  
*Int. J. Dev. Biol.* (2007) 51: 117-122

**5 yr ISI Impact Factor (2013) = 2.879**

